662. *The Structure of* Sterculia setigera *Gum. Part I. An Investigation by the Method of Paper Partition Chromatography of the Products of Hydrolysis of the Gum.*

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Sterculia setigera gum, a partly acetylated acidic polysaccharide, has been shown to consist of the following sugars in the approximate proportions indicated: D-galactose (5 parts), L-rhamnose (5 parts), D-tagatose (1 part), D-galacturonic acid (8 parts), and traces of two other sugars. Partial hydrolysis of the gum gave a mixture of reducing sugars and a stable acidic portion (*A*) (equiv., 234); methylation of (*A*), followed by fractional distillation in a high vacuum, gave a complicated mixture of oligo-saccharides which, on hydrolysis, yielded 3 : 4-dimethyl L-rhamnose, 2 : 3 : 6-trimethyl D-galactose, 2 : 3 : 4-trimethyl D-galacturonic acid, and 2 : 4(or 3 : 4)-dimethyl D-galacturonic acid. The significance of these results is discussed.

The tree *Sterculia setigera,* which occurs in tropical West Africa, forms on its bark a gum which is marketed in the form of hard brown amorphous nodules. This gum absorbs large quantities of water, swelling to many times its original size and giving a viscid gel, indistinguishable in appearance from solutions of other plant gums and mucilages. Like many of these materials it contains a high proportion of uronic acid residues.

Sterculia setigera gum, a sample of which was supplied to us by Sir John Simonsen, F.R.S., Director of Research to the Colonial Products Research Council, has not hitherto been described, but gums from other trees of the Sterculiaceæ family, namely *Sterculia urens* (Hirst, Jones, and Woods, unpublished results), *Sterculia tormentosa* (Beauquesne, *Compt. rend.*, 1946, **222**, 1056) and *Cochlospermum gossypium* (Robinson, *J.*, 1906, **89**, 1496; Dunstan, Hirst, and Jones, unpublished results) have received some attention. Karaya gum, the dried exudation of *Sterculia urens,* is of considerable commercial value as a substitute for the more expensive gum tragacanth in the textile, food, cosmetic, and other industries. It is clear from the preliminary investigations that there is a striking similarity in the composition of the various *Sterculia* gums, since they all occur as partly acetylated derivatives (acetyl content, *ca.* 16%) and, after purification, the acidic polysaccharides (acetyl-free) have an equivalent weight of about 400. Furthermore, in each case D-galacturonic acid, D-galactose, and L-rhamnose are the major constituents of the gums. *Sterculia setigera* gum shows a close relationship to these materials in that the natural product contains 15·5% of acetyl and the equivalent weight of the purified material is of the order of 370—400.

The carbohydrate material of each of three separate nodules of *Sterculia setigera* gum was purified by repeated precipitation by alcohol from acidic aqueous solutions, the final products

being obtained as colourless fibres. The optical rotatory powers, the equivalent weights, the uronic anhydride contents, and the sugars produced on hydrolysis (as examined by paper partition chromatography) were similar for each of the nodules, but the differences were sufficient to show that the composition of the gum may be variable.

The gum is extremely resistant to hydrolysis by mineral acids, such drastic conditions being required for complete scission that the reaction is accompanied by much degradation of the monosaccharides. The non-acidic reducing sugars produced were found, on examination on the paper chromatogram (Partridge, *Nature*, 1946, **158**, 270; Flood, Hirst, and Jones, *J.*, 1948, 1679; Forsyth, *Nature*, 1948, **161**, 239), to consist of equimolecular proportions of galactose and rhamnose, together with traces of two ketoses which occupied the same positions on the paper chromatogram as rhamnoketose and tagatose. Ketoses are very sensitive to acid and would have been largely destroyed under the conditions necessary for the complete hydrolysis of the gum. Partial hydrolysis of the gum, however, gave, with little degradation, a mixture of sugars and a stable acidic fraction (A, see Experimental section), the properties of which corresponded to those of a trisaccharide containing two uronic acid units and one sugar unit. After separation from the acidic fraction (A), the non-acidic reducing sugars were analysed by paper partition chromatography, and evidence was obtained for the presence of the following sugars in the proportions indicated: galactose (7 parts), tagatose (2 parts), rhamnose (5 parts), and rhamnoketose (traces). The mixture of sugars was separated by partition chromatography on a column of cellulose using *n*-butanol saturated with water as the mobile phase (Hough, Jones, and Wadman, this vol., p. 2511); the degree of separation may be judged from the following table, which shows the percentage yields of the components:

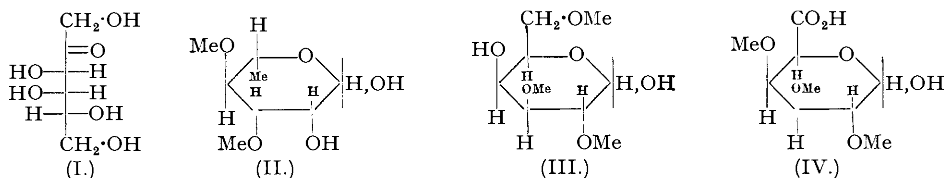
Fraction.	% Yield.	R_G values of the components.	Sugars present.
1	0·9	0·38	Rhamnoketose (?)
2	25·8	0 30	L-Rhamnose
3	8·2	0·17; 0·13	D-Tagatose; xylose (?)
4	23·3	0·17; 0·13; 0·07	D-Tagatose; xylose (?); D-galactose
5	40·0	0·07	D-Galactose

Fractions 2 and 5 crystallised spontaneously on removal of the solvent, and pure specimens of L-rhamnose hydrate and D-galactose were obtained. Fraction 1 contained a ketose, which displayed the same properties as rhamnoketose on the paper chromatogram, but further evidence is necessary for its conclusive identification. Two sugars were present in Fraction 3—an aldose and a ketose. The aldose was present only in small quantity and was not detected in the hydrolysis products of the gum, before this experiment, owing to the small quantity present. This aldose moved to the same position as xylose on the paper chromatogram, but this evidence is only indicative and further information is necessary for its conclusive identification. D-Tagatose (D-galactoketose) (I) was obtained in crystalline form after bromine oxidation of the aldose to the aldonic acid and removal of the latter by use of Amberlite resin IR4B. This sample of D-tagatose was identical with the synthetic specimen, kindly supplied by Professor T. Reichstein. D-Tagatose has not hitherto been reported as occurring in Nature and this is the first instance in which a ketose has been detected amongst the products of hydrolysis of a plant gum. A preliminary account of the identification of D-tagatose was published elsewhere (*Nature*, 1949, **163**, 177). The possibility of D-tagatose arising from D-galactose by epimerisation during the neutralisation procedure, which involves treatment with barium carbonate, was ruled out by removing the acid with an ion-exchange resin, Amberlite IR4B, leaving a neutral solution; concentration of this solution gave a syrup in which D-tagatose, D-galactose, and L-rhamnose were detected. It is considered that the tagatose is a constituent of the polysaccharide and not an artefact.

The acidic fraction (A) (equiv., 234) was extremely resistant to acid hydrolysis and required prolonged heating (24 hours) with 2N-sulphuric acid for its complete scission. Such drastic treatment results in loss of both uronic acid and non-acidic sugars. The uronic acid, isolated as the barium salt, was identified as D-galacturonic acid. In this respect, *Sterculia setigera* gum is similar to the plant mucilages and to gum tragacanth (James and Smith, *J.*, 1945, 749) rather than to the plant gums hitherto examined (cf. Hirst, this vol., p. 530).

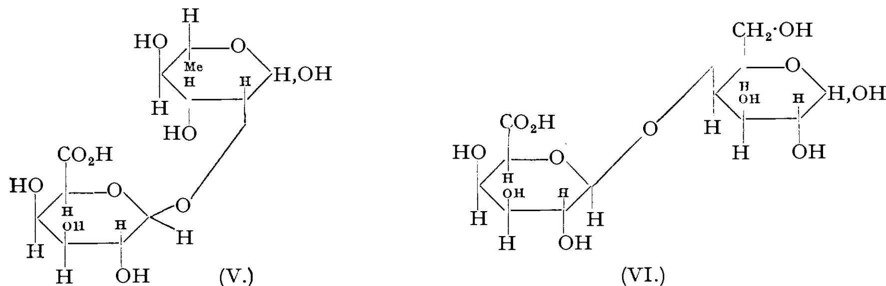
Whilst it is not yet possible to give an accurate estimate of the composition of the gum in view of the drastic conditions necessary for hydrolysis, the analytical data for the purified gum, considered in conjunction with the results obtained on hydrolysis of the gum, indicate that the following monosaccharides are present approximately in the proportions indicated: D-galactose (5 parts), L-rhamnose (5 parts), D-tagatose (1 part), and D-galacturonic acid (8 parts), with traces

of two other sugars, possibly xylose and rhamnoketose. This corresponds to an equivalent weight of 389.



An essential step towards the elucidation of the structure of the gum is the investigation of the stable residue which remains after partial hydrolysis of the gum. This material was therefore isolated and converted into the fully methylated derivative which could be purified by distillation in a high vacuum. The distillate consisted largely of material, the analytical data for which corresponded to those required for (*a*) a trisaccharide containing two uronic acid units and one sugar or (*b*) a mixture of disaccharides composed of a biuronoside (1 part) and an aldobiuronic acid (2 parts). The boiling point of the fraction strongly suggests that the latter view is correct. Hydrolysis of the fully methylated material yielded a mixture of reducing sugars composed of an acidic and a neutral fraction. The non-acidic sugars consisted of 3 : 4-dimethyl L-rhamnose (86%) (II) and a small quantity of 2 : 3 : 6-trimethyl D-galactose (14%) (III). These derivatives were completely separated on a column of cellulose by partition chromatography, using a mixture of butanol and light petroleum as the mobile phase. 3 : 4-Dimethyl L-rhamnose was identified as the crystalline sugar (Bott, Haworth, and Hirst, *J.*, 1930, 1395; Levene and Kreider, *J. Biol. Chem.*, 1937, **120**, 602). The galactose derivative was recognised after oxidation, with bromine water, to crystalline 2 : 3 : 6-trimethyl D-galactonofuranolactone (Oldham and Bell, *J. Amer. Chem. Soc.*, 1938, **60**, 323).

The analytical figures for the methylated uronic acids produced on hydrolysis of the methylated material corresponded approximately to those required for equimolecular proportions of tri- and di-methyl galacturonic acids. This mixture was separated on a column of cellulose, using a mixture of butanol, acetic acid, and water as the mobile phase. The separation was not absolute but pure specimens of 2 : 3 : 4-trimethyl (IV) (45% of the total solids) and dimethyl D-galacturonic acid (31%) were obtained. Very small quantities of two other uronic acid derivatives, the positions of which corresponded to 2 : 3-dimethyl and 2-methyl galacturonic acid on the paper chromatogram, were obtained. The uronic acids were detected on the paper chromatogram by spraying it with an alcoholic solution of trichloroacetic acid and aniline and then heating the chromatogram, intense red spots being thus formed. These products arise either from incomplete methylation or (less probably) from the demethylation of the more fully methylated uronic acids during methanolysis. The dimethyl D-galacturonic acid was obtained as a syrup which with cold methanolic hydrogen chloride did not exhibit a downward change in optical rotation, the presence of a methoxyl group on $C_{(4)}$ being thus indicated. (A solution of 2 : 3-dimethyl D-galacturonic acid in cold methanolic hydrogen chloride changes from a positive to a negative value in optical rotation.) This suggests that it is either 3 : 4- or 2 : 4-dimethyl D-galacturonic acid, but neither of these materials has been described in the literature and direct comparison was not possible. From the evidence cited above, it would appear that the products obtained by partial hydrolysis of the gum contained at least three disaccharides :



(1) 2-D-galacturonosido-L-rhamnose (V) in which both residues are present in the pyranose form; this aldobiuronic acid has been found amongst the hydrolysis products of linseed mucilage (Tipson, Christman, and Levene, *J. Biol. Chem.*, 1939, **128**, 609) and slippery elm mucilage

(Gill, Hirst, and Jones, *J.*, 1939, 1469); (2) D-galacturonosido-D-galacturonic acid, in which a pyranose D-galacturonic acid residue is united by its reducing group to a hydroxyl group at either $C_{(3)}$ or $C_{(2)}$ of another pyranose D-galacturonic acid residue; and (3) a small quantity of an aldobiuronic acid, D-galacturonosido-D-galactose (VI), in which the pyranose form of the uronic acid is linked through its reducing group to a hydroxyl group at $C_{(4)}$ of the galactose residue (on the assumption that the galactose occurs in the pyranose form).

Experimental.

All boiling points are recorded as bath temperatures, and optical rotations are measured in aqueous solution, unless otherwise stated.

Purification and Properties of Sterculia setigera *Gum.*—*Sterculia setigera* gum occurs as the partly acetylated derivative of the inorganic salt of an acidic polysaccharide, the acidity being due to uronic acid residues (Found, for dried material: sulphated ash, 12·8; OMe, nil; N, nil; S, nil; Ac, 15·5%). The gum absorbs large quantities of water, swelling to many times its original size to form a bulky jelly which is neutral and does not reduce Fehling's solution. Three nodules of gum were selected and purified in the following manner. The nodule was dried in a steam-oven for 4 hours and then powdered and dissolved with stirring (24 hours) in 5% sodium hydroxide solution. Bark and other extraneous material were removed at the centrifuge, and the clear, brown, viscous solution was decanted. The solution was chilled and acidified by the careful addition of concentrated hydrochloric acid, and the acidic polysaccharide was isolated by pouring the pale yellow mobile solution, with stirring, into 4 volumes of absolute alcohol. The fibrous precipitate was removed on a spatula and was immediately dissolved, with stirring (24 hours), in 20% aqueous alcohol. Subsequently, the polysaccharide was reprecipitated by pouring the solution into absolute alcohol. The precipitate was dried to constant weight at 60° under reduced pressure over phosphoric oxide. The acidic polysaccharide gave negative tests for chloride ions and acetyl groups; it was difficultly soluble in water, but readily dissolved in aqueous sodium hydroxide. The sodium salt of the polysaccharide was non-reducing and with copper sulphate solution gave an insoluble copper salt. Each of the three polysaccharide samples (2·5 g. each) was hydrolysed with N-sulphuric acid (100 ml.) on the boiling-water bath for 20 hours, the reaction being followed polarimetrically. After hydrolysis, the solutions were neutralised with barium carbonate and filtered. The filtrates were evaporated under reduced pressure at 40° and the syrups so obtained were examined on the paper chromatogram. The results suggested the presence in each hydrolysate of a uronic acid (R_G 0·00), galactose (R_G 0·07), tagatose (R_G 0·13), rhamnose (R_G 0·30), and rhamnoketose (R_G 0·38).

Determination of the Sugars produced on Complete Hydrolysis of the Acidic Polysaccharide.—The purified gum (20 mg.) was hydrolysed with 2N-sulphuric acid in a sealed tube at 100° for 24 hours. The solution was neutralised by the use of Amberlite resin IR4B, filtered, and evaporated to a thin syrup. The neutral reducing sugars present in this were separated on a paper chromatogram, and each sugar was determined by use of Somogyi's micro-copper reagent (Flood, Hirst, and Jones, *loc. cit.*). The strips of filter paper, each containing one of the separated sugar components, contained respectively galactose 1·31 mg., tagatose a trace, rhamnose 1·16 mg., and rhamnoketose a trace. The major constituents, galactose and rhamnose, were therefore present in equimolecular proportions.

Graded Hydrolysis of the Gum.—Dry *Sterculia* gum (25 g.) was heated with 0·05N-sulphuric acid (500 ml.) at 90—95°, the reaction being followed polarimetrically: $[\alpha]_D$ +66° (3 hours), +70° (10 hours), +80° (13 hours), +80° (14 hours), and +78° (15 hours). The solution was then cooled and the liberated acetic acid removed by continuous extraction with ether. The solution was neutralised by the addition of barium carbonate and filtered, and the filtrate was evaporated under reduced pressure at 40°. The residue (*A*) (25 g.) was extracted with boiling methyl alcohol (4 × 250 ml.). The insoluble barium salts (17 g.) were collected and dried.

Analysis and Identification of the Neutral Reducing Sugars from (A).—The combined methanolic extracts were concentrated under reduced pressure to a syrup {5·8 g.; $[\alpha]_D$ +40·5° (*c*, 3·3); sulphated ash, 7·2%; Ac, nil}. Quantitative analysis of a portion of the syrup by paper partition chromatography indicated that galactose, tagatose, and rhamnose were present in the molecular proportions 7 : 2 : 5, and that only traces of rhamnoketose were present. The galactose and rhamnose were also determined as the phenylmethylhydrazone and the benzoylhydrazone respectively (Hirst, Jones, and Woods, *J.*, 1947, 1048). A portion (0·143 g.) of the syrup in water (3 ml.) gave galactose phenylmethylhydrazone, m. p. 185° (decomp.) (0·083 g., ≡0·061 g. of galactose, corresponding to 43% of the syrupy mixture of sugars). In addition, another portion of the syrup crystallised on storage; D-galactose, isolated after draining of the material on a tile and recrystallised from methyl alcohol, had m. p. and mixed m. p. 161°, $[\alpha]_D$ +81·7° (equilibrium; *c*, 0·42). To another portion (0·83 g.) of the syrup in water (1·5 ml.) a saturated solution of benzhydrazide in alcohol (7·5 ml.) was added. Rhamnose benzoylhydrazone separated, having m. p. 183° (decomp.) (0·056 g., corresponding to the presence of 34% of rhamnose in the syrup).

A portion (1·6 g.) of the sugar mixture was separated by partition chromatography on a column of cellulose, with *n*-butanol, saturated with water, containing a little ammonia as the mobile phase. The eluate was fractionated as described by Hough, Jones, and Wadman (*loc. cit.*). The eluate was divided into the following five fractions, and the solvent was removed by evaporation under reduced pressure at 40°.

(1) 15 Mg., $[\alpha]_D$ +12·0° (*c*, 0·8), R_G 0·38. This contained a ketose which in view of its high R_G value is, in all probability, a pentose derivative. It moved to the same position on the paper chromatogram as rhamnoketose and gave an intense red colour when heated with naphtharesorcinol and trichloroacetic acid.

(2) 414 Mg., $[\alpha]_D$ +9·4° (*c*, 1·0), R_G 0·30. This crystallised spontaneously on removal of the solvent, giving L-rhamnose hydrate, m. p. 95°, $[\alpha]_D$ +9·4° (equilibrium; *c*, 1·0); the m. p. of the anhydrous substance was 124°, undepressed on admixture with an authentic specimen.

(3) 145 Mg., $[\alpha]_D$ +8·0° (*c*, 1·1). This contained D-tagatose (R_G 0·13) and another sugar (R_G 0·17) which behaves as xylose on the paper chromatogram. The mixture contained 25·4% of aldose, as

determined by oxidation with alkaline hypoiodite. The aldose was removed in the following manner. A portion of the fraction (120 mg.) was oxidised with bromine water for 24 hours at room temperature. The bromine was then removed by aëration, the solution was neutralised with silver carbonate, and the insoluble silver salts were filtered off. Silver was removed by passage of hydrogen sulphide, followed by filtration. The aldonic acid was then removed by use of Amberlite resin IR4B, and the neutral solution was evaporated to a syrup (69 mg.). D-Tagatose was obtained crystalline when the syrup was seeded with a synthetic specimen {m. p. and mixed m. p. 133—135°, $[\alpha]_D^{17}$ $-2°$ (*c*, 0·85)}. Proof of identity was given by *X*-ray analysis for which we are indebted to Dr. T. Malkin. Further confirmation was obtained by the formation of D-galactosazone (m. p. and mixed m. p. 175°).

(4) 373 Mg., $[\alpha]_D$ $+33·8°$ (*c*, 1·1). This contained at least three sugars which moved at the same rate as galactose (R_G 0·07), tagatose (R_G 0·13), and xylose (R_G 0·17) on the paper chromatogram.

(5) 640 Mg., $[\alpha]_D$ $+80·5°$ (*c*, 0·5), R_G 0·07. This crystallised spontaneously after removal of the solvent, giving D-galactose, m. p. and mixed m. p. 161°, $[\alpha]_D$ $+80·5°$ (equilibrium; *c*, 0·5).

Analysis and Hydrolysis of the Barium Salts obtained from (A).—The barium salts were obtained as a white amorphous powder, easily soluble in water and showing $[\alpha]_D$ $+59°$ (*c*, 2·2) (Found: Ba, 22·8%; Ac, nil). Preliminary experiments showed that the barium salts were extremely resistant to hydrolysis, requiring the use of 2N-sulphuric acid for their complete scission. The barium salts (2·5 g.) were heated at 90—95° with 2N-sulphuric acid (100 ml.) for 24 hours, the reaction being followed polarimetrically: $[\alpha]_D$ $+56°$ (initial), $+33°$ (4 hours), $+19°$ (14 hours), $+16°$ (17 hours), $+12°$ (19 hours), and $+12°$ (24 hours). The reaction was accompanied by degradation of the sugars, and after 14 hours it was necessary to treat a small sample with charcoal before each polarimetric observation. The brown solution was neutralised with barium carbonate, filtered, and evaporated in the usual manner. The residue (*B*) (1·2 g.) was extracted with boiling methyl alcohol (3 × 50 ml.), and the insoluble barium salts were collected and dried.

Analysis and Identification of the Neutral Reducing Sugars from (B).—The syrup {0·86 g.; $[\alpha]_D$ $+11·6°$ (*c*, 0·85)} obtained on evaporation of the methanolic extracts was examined chromatographically, galactose, rhamnose, and traces of tagatose and rhamnoketose being detected. In a quantitative experiment, the zones of paper holding these sugars were found to contain respectively 0·216 mg. of galactose and 0·515 mg. of rhamnose. These sugars were therefore present in the molecular ratio 2 : 5. D-Galactose was determined and characterised as the phenylmethylhydrazone derivative, m. p. 185° (decomp.), the yield of which corresponded to 13·5% of galactose in the syrup. The methyl pentose content of the syrup was determined by oxidation with sodium periodate and determination of the acetaldehyde produced (method of Nicolet and Shinn, *J. Amer. Chem. Soc.*, 1941, **63**, 1456). The yield of acetaldehyde corresponded to 19% of rhamnose in the syrup.

Analysis and Identification of the Barium Salts from (B).—The barium salts (0·30 g.) showed $[\alpha]_D$ $+21°$ (*c*, 1·0) and contained some degraded material (Found: Ba, 33·6. A barium hexuronate requires Ba, 26·2%). Barium D-galacturonate shows $[\alpha]_D$ $+25·1°$ (*c*, 1·4) (Ohle and Berend, *Ber.*, 1925, **58**, 2585). The barium salts (0·2 g.) were heated with 20% nitric acid (3 ml.) on a steam-bath until the volume had diminished by one-third. Next morning, crystals of mucic acid had separated; recrystallised from water these had m. p. and mixed m. p. 216° (decomp.) (Found: equiv., 104. Calc. for $C_6H_{10}O_8$: equiv., 105). A brick-red precipitate was formed when a solution of the barium salt was warmed with a drop of basic lead acetate solution. This test is not given by glucuronic or mannuronic acid and is claimed to be specific for galacturonic acid (Ehrlich, *Ber.*, 1932, **65**, 352).

Methylation of the Barium Salts from (A).—The barium salts (12 g.) were dissolved in water (50 ml.), and methyl sulphate (50 ml.) and 40% sodium hydroxide solution were added dropwise during 5 hours with vigorous stirring. The reaction mixture then no longer reduced Fehling's solution. Sodium hydroxide solution (40%; 200 ml.) and methyl sulphate (130 ml.) were added dropwise during 3 hours, with continuous stirring, and stirring was continued overnight. The solution was chilled, rendered slightly alkaline by the cautious addition of 25% sulphuric acid, and then evaporated on the steam-bath to a thin paste. The residue was methylated once more by the addition of methyl sulphate (180 ml.) and 40% sodium hydroxide solution (300 ml.). After methylation, the solution was chilled, acidified with 25% sulphuric acid, and extracted 3 times with chloroform (100 ml. portions). The combined extracts were dried (Na_2SO_4) and were then evaporated under reduced pressure to a syrup (8 g.) (Found: OMe, 42·4%). The product was etherified by two treatments with methyl iodide and silver oxide. The product (6·9 g.) (Found: OMe, 47·1%) was distilled at 3×10^{-4} mm., giving fractions as follows: (i) (0·548 g.), b. p. 140—155°, $[\alpha]_D^{22}$ $+13°$ (*c*, 0·53 in methanol) [Found: OMe, 54·2%; equiv. (by quantitative alkaline hydrolysis), 346. The methyl ester of trimethyl methylgalacturonoside requires OMe, 58·7%; equiv., 264]; (ii) (0·148 g.), b. p. 220—230°, $[\alpha]_D^{22}$ $+23·1°$ (*c*, 0·65 in methanol), a viscous yellow syrup (Found: OMe, 50·5%; equiv., 309); (iii) (0·509 g.), b. p. 230—250°, $[\alpha]_D^{22}$ $+61°$ (*c*, 0·75 in methanol), a viscous orange syrup (Found: OMe, 49·7%; equiv., 307); (iv) (0·733 g.), b. p. 250—260°, $[\alpha]_D^{22}$ $+65°$ (*c*, 1·0 in methanol), a viscous orange syrup (Found: OMe, 47·8%; equiv., 308); (v) (0·375 g.), b. p. 260—270°, $[\alpha]_D^{22}$ $+63°$ (*c*, 0·80 in methanol), a viscous orange syrup (Found: OMe, 48·0%; equiv., 304); and (vi) (still residue) (3·82 g.), a friable brown solid, $[\alpha]_D^{22}$ $+94°$ (*c*, 1·0 in chloroform) (Found: OMe, 44·2%; equiv., 360).

The constants of fractions (ii)—(v) are very close to those required for either (*a*) a methylated trisaccharide consisting of two galacturonic acid units and one rhamnose unit (Calc.: OMe, 47·3%; equiv., 328) or (*b*) a mixture containing one molecular proportion of a diuronide, consisting of two galacturonic acid units, and two molecular proportions of an aldobiuronic acid consisting of one galacturonic acid unit and one rhamnose unit (Calc.: OMe, 50·2%; equiv., 339).

Examination of fractions. (i)—(vi). (i) A portion (0·17 g.) was dissolved in N-sulphuric acid (10 ml.) and heated at 90—95° for 10 hours: $[\alpha]_D$ $+38°$ (initial value), $+45°$ (3 hours), and $+47°$ (5·5 hours; constant value). The solution was neutralised by titration with N-sodium hydroxide solution, and the neutral methylated sugars were removed by continuous extraction with chloroform for 8 hours. On evaporation the chloroform extract yielded a syrup (0·056 g.) which showed $[\alpha]_D^{21}$ $+48°$ (*c*, 1·0) (Found: OMe, 43·6%). The syrup was investigated by partition chromatography on filter paper and appeared

to contain 2 : 3 : 4-trimethyl rhamnose (R_G 1·01) and 2 : 3 : 4 : 6-tetramethyl galactose (R_G 0·88). These materials arose from rhamnose and galactose, small quantities of which had not been completely extracted from the barium salt from (*A*). The neutral aqueous solution was acidified, and extraction with chloroform was continued for 8 hours. The chloroform extract was dried ($MgSO_4$) and concentrated under reduced pressure to a syrup (0·027 g.) which showed $[\alpha]_D^{22}$ +22° (*c*, 0·54) (Found: OMe, 39·9%). Calc. for $C_9H_{16}O_7$: OMe, 39·4%). The rotation of the syrup indicated the presence of 2 : 3 : 4- and 2 : 3 : 5-trimethyl D-galacturonic acids. The latter probably arose from the transformation of a pyranose to a furanose ring-form during the hydrolysis of the gum (cf. Luckett and Smith, *J.*, 1940, 1106).

(ii)—(vi). Each fraction (50 mg.) was heated at 100° with 4% methanolic hydrogen chloride (1 ml.) in a sealed tube for 15 hours. The resulting methylglycosides were then hydrolysed with 4% hydrochloric acid (5 ml.) for 3 hours at 100°. After neutralisation with Amberlite resin IR4B, the neutral reducing sugars were investigated by paper partition chromatography. The hydrolysates from fractions (ii)—(v) each showed spots on the paper chromatogram at positions R_G 0·84 and R_G 0·71, corresponding to 3 : 4-dimethyl rhamnose and 2 : 3 : 6-trimethyl galactose. The paper chromatogram obtained for fraction (vi) showed four spots—at R_G 0·88, 0·71, 0·51 and 0·44—and was identical in appearance with a paper chromatogram of the methylated sugars obtained on hydrolysis of methylated *Sterculia* gum, details of which will appear in Part II. Fractions (ii) and (vi) were not further investigated. From the results cited above for fractions (iii)—(v) it was concluded that there was no essential difference in their properties and they were therefore combined. The combined fractions (1·2 g.) were dissolved in 4% methanolic hydrogen chloride and heated at 100° in a sealed tube for 24 hours. The resultant methylglycosides were hydrolysed with 4% hydrochloric acid in the usual way, and the cooled solution was neutralised by the addition of silver carbonate. The insoluble silver salts were removed by filtration, and the silver ions removed by the passage of hydrogen sulphide followed by filtration. The acidic filtrate was neutralised by the addition of barium carbonate. Excess of barium carbonate was then removed by filtration, and the neutral solution was evaporated to dryness. The residue (*C*) (1·1 g.) was extracted 3 times with boiling ether (50 ml. portions). The insoluble barium salts (0·834 g.) were collected and dried.

Identification of the Methylated Sugars from (C).—The syrup (0·264 g.) obtained when the ethereal extracts were concentrated under reduced pressure showed $[\alpha]_D^{22}$ +56° (*c*, 0·696), n_D^{14} 1·4660 (Found: OMe, 33·5%. Calc. for dimethyl rhamnose : OMe, 32·3%). A small portion of the syrup was examined on the paper chromatogram and, after development in the usual manner, was found to contain mainly 3 : 4-dimethyl rhamnose (R_G 0·84) together with a small quantity of 2 : 3 : 6-trimethyl galactose (R_G 0·71). The syrup was fractionated by partition chromatography on a column of cellulose, using a mixture of light petroleum (b. p. 100—120°) (60%) and *n*-butanol (40%) as the mobile phase. The eluate was divided into approx. 5-ml. portions by an automatic device which changed the receiver every 10 minutes. The dimethyl rhamnose was completely separated from the trimethyl galactose, and two portions (*D*) and (*E*) were obtained which were evaporated under reduced pressure at 40°.

Fraction (*D*) was purified by dissolution in water, filtration, and evaporation, to remove a little cellulosic material. The residue was dissolved in ether, a little insoluble material was filtered off, and the ether was removed by evaporation. The syrup (0·198 g.) crystallised spontaneously as fine needles which were recrystallised from ether–light petroleum and identified as 3 : 4-dimethyl L-rhamnose {m. p. 98—99°; $[\alpha]_D$ +24° (*c*, 0·54) (10 minutes) ⟶ +18·5° (16 hours; equilibrium value); R_G 0·84} (Found : OMe, 32·5. Calc. for $C_8H_{16}O_5$: OMe, 32·3%). The identity of the crystals was confirmed by periodate oxidation, one mole of sodium periodate being consumed by 200 g. of dimethyl rhamnose without formation of acetaldehyde (192 g. of 3 : 4-dimethyl rhamnose should consume 1 mole of sodium periodate).

Fraction (*E*) {0·033 g.; R_G 0·71; $[\alpha]_D$ +76° (*c*, 1·2)} (Found : OMe, 35·7. Calc. for 2 : 3 : 6-trimethyl D-galactose : OMe, 41·8%) was examined in the same manner as fraction (*D*). The methylated sugar was oxidised with bromine water at room temperature for 24 hours. Excess of bromine was removed by aëration; the solution was then neutralised with silver carbonate and filtered before and after passage of hydrogen sulphide. On evaporation, crystals of 2 : 3 : 6-trimethyl D-galactofuranolactone were obtained, having m. p. and mixed m. p. 97—98° (Found : OMe, 40·6. Calc. for $C_9H_{16}O_6$: OMe, 42·4%).

Identification of the Barium Salts from (C).—The residue consisted largely of a mixture of the barium salts of tri- and di-methyl galacturonic acids (Found : OMe, 23·3; Ba, 20·6. Calc. for an equimolecular mixture: OMe, 26·0; Ba, 23·2%). The barium salts (0·8 g.) were dissolved in water (50 ml.), and the theoretical quantity of N-sulphuric acid was added to precipitate the barium. After filtration, the solution was evaporated to a viscid syrup (0·603 g.). A small portion of the syrup was examined on the paper chromatogram, using the top layer of a mixture of *n*-butanol (40%), acetic acid (10%) and water (50%) as the mobile phase. The positions of three methylated uronic acid derivatives were revealed by spraying the paper chromatogram with an alcoholic solution of aniline (1%) and trichloroacetic acid (5%) and then heating it. The uronic acids gave rise to intense red spots at R_G 0·66 and R_G 0·505 and to a faint red spot at R_G 0·175, corresponding to tri-, di-, and mono-methyl galacturonic acid, respectively.

The methylated uronic acids were separated by partition chromatography on a column of cellulose, using a mixture of butanol (95%) and acetic acid (5%), saturated with water, as the mobile phase. The eluate was collected in 5-ml. portions by changing the receiver every 15 minutes and, after examination on sheet-paper chromatograms, it was divided into 6 fractions (*F*)—(*K*) (see table below). Before

Fraction.	Weight (mg.).	R_G values of the components.*	$[\alpha]_D^{14}$.	D-Galacturonic acid derivatives.
F	242	0·62	+104° (*c*, 0·46)	2 : 3 : 4-Trimethyl
G	90	0·62, 0·50	+99 (*c*, 0·9)	2 : 3 : 4-Trimethyl and dimethyl
H	165	0·50	+93 (*c*, 1·13)	Dimethyl
I	31	0·50, 0·35	+45 (*c*, 1·05)	Dimethyl
J	13	0·35	+40 (*c*, 0·42)	2 : 3(?)-Dimethyl
K	trace	0·15	—	Monomethyl

* With butanol–acetic acid–water as the mobile phase.

evaporation under reduced pressure at 40°, water (50 ml. to every 100 ml. of eluate) was added to each fraction in order to minimise esterification of the uronic acids. After evaporation, each fraction was heated with N-sulphuric acid for 3 hours, neutralised by the addition of barium carbonate, filtered, and evaporated. The insoluble barium salts were dissolved in water, barium was removed by use of Amberlite resin IR-100, and the solution was evaporated to dryness under reduced pressure.

Analysis and identification of fractions (*F*)—(*K*). Fraction (*F*) crystallised spontaneously and was identified as 2 : 3 : 4-trimethyl D-galacturonic acid monohydrate, m. p. 98—99°, $[\alpha]_D^{14}$ +120° ⟶ 104° (equilibrium value; *c*, 0·46) (Found : OMe, 36·8%; equiv., 260. Calc. for $C_9H_{16}O_7,H_2O$: OMe, 36·7%; equiv., 254). A portion (0·1 g.) was oxidised with nitric acid (1 ml.; *d* 1·3) at 100° for 4 hours. The reaction mixture was then diluted with water (5 ml.) and evaporated under reduced pressure at 40°. The residue was redissolved in water (5 ml.) and evaporated again. After this evaporation had been repeated 20 times, the product (0·074 g.) was free from nitric acid and showed $[\alpha]_D^{17}$ +49° (*c*, 0·79 in acetone). The syrup was dissolved in 4% methanolic hydrogen chloride (5 ml.) and boiled under reflux for 12 hours. The hydrogen chloride was removed with silver carbonate, and the filtrate was evaporated under reduced pressure to a pale yellow syrup (0·06 g.) which crystallised on nucleation with a specimen of the dimethyl ester of 2 : 3 : 4-trimethyl mucic acid. After removal of the syrup on a tile, the crystals were recrystallised from ether–light petroleum; they then had m. p. 99—100°, $[\alpha]_D^{14}$ +30·2° (*c*, 0·78), and did not depress the m. p. of an authentic specimen of the dimethyl ester of 2 : 3 : 4-trimethyl mucic acid (Found : OMe, 54·6%. Calc. for $C_{11}H_{20}O_8$: OMe, 55·4%).

Fraction (*G*) (Found : OMe, 25·6%) was an intermediate fraction which contained the same substances as were present in (*F*) and (*H*), as indicated by paper partition chromatography.

Fraction (*H*) contained a dimethyl galacturonic acid which awaits identification (Found : OMe, 25·3%; equiv., 218. Calc. for $C_8H_{14}O_7$: OMe, 27·9%; equiv., 222). A portion (0·0206 g.) was dissolved in cold methanolic hydrogen chloride (3 ml.), and the optical rotation of the solution observed : $[\alpha]_D$ +46·5° (15 minutes), +78·5° (4 hours), and +93° (24 hours) (under these conditions, 2 : 3-dimethyl D-galacturonic acid is converted into the furanose derivative which has a negative optical rotation).

Fraction (*I*) contained a mixture of dimethyl galacturonic acids (Found : OMe, 24·5%; equiv., 220. Calc. for $C_8H_{14}O_7$: OMe, 27·9%; equiv., 222). Fractions (*I*) and (*J*) were present only in very small quantity and possibly arose from demethylation during methanolysis. On the paper chromatogram they gave spots in the same positions as 2 : 3-dimethyl galacturonic acid and 2-methyl galacturonic acid respectively.

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