247. *The Structure of* Sterculia setigera *Gum. Part II. An Investigation by the Method of Paper Partition Chromatography of the Products of Hydrolysis of the Methylated Gum.*

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Sterculia setigera gum has been converted into its fully methylated derivative, which on hydrolysis gives 2 : 3 : 4 : 6-tetramethyl D-galactose (6 parts), 2 : 3 : 6-trimethyl D-galactose (3 parts), 3 : 4(?)-dimethyl L-rhamnose (4 parts), 2- and/or 3-methyl L-rhamnose (3 parts), 2 : 6-dimethyl D-galactose (traces), L-rhamnose (traces), and 2-methyl D-galacturonic acid (16 parts), in the approximate proportions indicated.

In Part I (Hirst, Hough, and Jones, *J.*, 1949, 3145), it was shown that *Sterculia setigera* gum contains the following sugars in the approximate proportions indicated: D-galactose (5 parts), D-tagatose (1 part), L-rhamnose (5 parts), and D-galacturonic acid (8 parts), with traces of two other sugars, possibly rhamnoketose and xylose. Partial hydrolysis of the gum gave a mixture of reducing sugars and a degraded material which contained the uronic acid. Methylation of this degraded material followed by fractional distillation of the fully methylated product gave a mixture of disaccharides containing an aldobiuronic acid designated 2-D-galactopyruronosido-L-rhamnopyranose, a biuronoside containing two D-galactopyruronic acid units joined by either a 1 : 2- or a 1 : 3-linkage, and a small quantity of another aldobiuronic acid, 4-D-galactopyruronosido-D-galactose. Further information concerning the mode of linkage of the various component sugars in *Sterculia setigera* gum has now been obtained from an examination of the products of hydrolysis of the fully methylated polysaccharide. The methylated gum, which was substantially homogeneous since no portions differing materially from one another were obtained on fractionation, gave on hydrolysis a complex mixture of sugar derivatives. After separation of the methylated uronic acids, the neutral methylated reducing sugars were examined by paper partition chromatography. The chromatogram showed five spots, the rate of movement of which corresponded to **2 : 3 : 4 : 6**-tetramethyl galactose and **3 : 4**-dimethyl rhamnose (R_G, 0·88), **2 : 3 : 6**-dimethyl galactose (R_G, 0·71), methyl rhamnose (R_G, 0·57), 2 : 6-dimethyl galactose (R_G, 0·44), and rhamnose (R_G, 0·30). Methylated tagatose derivatives (see Part I) were looked

for but they were not detected after removal of the aldose derivatives by oxidation with bromine water and subsequent treatment with Amberlite resin IR-4B to eliminate aldonic acids. It appears, therefore, that the tagatose residues were destroyed during the methanolysis of the methyl derivative. The mixture of methylated sugars was separated into four components by partition chromatography on a column of cellulose by using light petroleum–butanol as the mobile phase (Hough, Jones, and Wadman, *J.*, 1949, 2511). The percentage yields of the fractions calculated from the weight of material introduced into the column are shown in the table.

Fraction.	Yield, %.	R_G value.	Methylated sugars present.
I	52	0·88	2 : 3 : 4 : 6-Tetramethyl galactose 3 : 4(?)-Dimethyl rhamnose
II	18	0·71	2 : 3 : 6-Trimethyl galactose
III	15	0·57	2- and/or 3-Methyl rhamnose
IV	3	0·44	2 : 6-Dimethyl galactose

Fraction I. This fraction contained tetramethyl galactose (60%) and dimethyl rhamnose (40%). 2 : 3 : 4 : 6-Tetramethyl D-galactose was identified as its well-characterised crystalline anilide. This mixture of methylated reducing sugars could not be resolved into its constituents by fractional distillation, and attempts to separate the anilides by fractional crystallisation were equally unsuccessful. An estimate of the amount of sodium periodate consumed by the distilled material indicated that the fraction contained approximately 24% of dimethyl rhamnose in which two adjacent hydroxyl groups are unsubstituted. 2 : 4-Dimethyl rhamnose will not be oxidised by periodate, and it has been shown that 2 : 3-dimethyl rhamnose is only oxidised to a small extent, about 0·1 mole of periodate being consumed per mole of sugar (Brown, Hough, and Jones, *J.*, 1950, 1125). On the other hand, 3 : 4-dimethyl rhamnose consumes 0·7 mole of sodium periodate per mole of sugar. It would appear, therefore, that the fraction contains some 35% of 3 : 4-dimethyl L-rhamnose, assuming that all the rhamnose derivatives possess a hydroxyl grouping on $C_{(5)}$. The isolation of 3 : 4-dimethyl L-rhamnose from the methylated degradation products of the gum (Hirst, Hough, and Jones, *loc. cit.*) shows that some at least of the rhamnose residues are linked through $C_{(1)}$ and $C_{(2)}$ and that the rhamnose is in the pyranose form. It is of interest to note that rhamnose has not hitherto been encountered in the furanose form in any natural product.

Fraction II. This consisted of 2 : 3 : 6-trimethyl D-galactose, which was recognised by its ability to form a furanose derivative with decrease of optical rotation in cold methanolic hydrogen chloride. After oxidation with bromine water it yielded crystalline 2 : 3 : 6-trimethyl γ-D-galactonolactone (Haworth, Hirst, and Stacey, *J.*, 1932, 2481).

Fraction III. The properties of this fraction corresponded to those of a monomethyl L-rhamnose which, after oxidation with bromine water, gave a syrupy γ-lactone. The sodium salt of the methyl rhamnonic acid was oxidised with sodium periodate with the formation of acetaldehyde. These two observations indicate that there must be a free hydroxyl group on $C_{(4)}$ and $C_{(5)}$ and it follows that the fraction contains either 2-methyl or 3-methyl L-rhamnose or both and that 4-methyl rhamnose is absent. The significance of the low yield of acetaldehyde (50%) produced on oxidation of the fraction with sodium periodate will be discussed in a later publication (cf., however, Bell, *J.*, 1948, 992, and Brown, Hough, and Jones, *loc. cit.*)

Fraction IV. This consisted of 2 : 6-dimethyl D-galactose (Oldham and Bell, *J. Amer. Chem. Soc.*, 1938, **60**, 323), and it was obtained in crystalline form on seeding with an authentic specimen.

The methylated uronic acid portion consisted mainly of 2-methyl D-galacturonic acid, which was recognised after oxidation with bromine water, followed by esterification with methanolic hydrogen chloride and conversion of the dimethyl ester into the crystalline diamide of 2-methyl mucic acid (Jones and Stacey, *J.*, 1947, 1340).

Owing to the difficulty encountered in the identification of the various methylated sugars, the following figures are only a provisional estimate of the approximate proportions of the sugar derivatives formed on hydrolysis of methylated *Sterculia setigera* gum : 2 : 3 : 4 : 6-tetramethyl D-galactose (6 parts), 3 : 4(?)-dimethyl L-rhamnose (4 parts), 2 : 3 : 6-trimethyl D-galactose (3 parts), 2- and/or 3-monomethyl L-rhamnose (3 parts), 2 : 6-dimethyl D-galactose (trace), rhamnose (traces), and 2-methyl D-galacturonic acid (16 parts). The 2 : 6-dimethyl galactose and rhamnose may result from either incomplete methylation of the polysaccharide or from demethylation of the more fully methylated sugars during methanolysis.

At this stage it is not possible to formulate a unique structure for this complex polysaccharide.

It is apparent, however, that *Sterculia setigera* gum contains the following residues linked at the positions indicated, the precise order of arrangement being as yet undefined,

Gal, 1....(I); ....4, Gal, 1....(II); ....2, Rh, 1....(III);

....4, Rh, 1...and/or....4, Rh 1....(IV); ...4, Gala, 1...(V),

2 3 3

: : :

where Gal = D-galactopyranose; Rh = L-rhamnopyranose; Gala = D-galacturonic acid. The presence of large amounts of end groups (I) and of triply linked galacturonic acid (V) in the polysaccharide illustrates the highly branched nature of the macro-molecule.

Experimental.

Unless otherwise stated the boiling points are bath temperatures and the optical rotations were measured in aqueous solution.

Methylation of Sterculia setigera *Gum.*—A thick gelatinous solution of the gum (30 g.) in 40% sodium hydroxide solution (700 ml.) (prepared by adding the powdered dry gum to the sodium hydroxide solution and stirring overnight) was methylated by the gradual addition of methyl sulphate (450 ml.) during 6 hours at room temperature and with stirring. The stirring was continued overnight. The solution, cooled in an ice-bath, was neutralised by the careful addition of 25% sulphuric acid and was then evaporated on a steam-bath. The resultant paste was stirred with 40% sodium hydroxide solution (200 ml.) in order to dissolve the partially methylated gum which was methylated again by the gradual addition of 40% sodium hydroxide solution (300 ml.) and methyl sulphate (320 ml.). The evaporation process was repeated and a third methylation carried out. The solution was then dialysed against a continuous stream of tap water until it was free from sulphate ions and was neutral in reaction. The neutral solution was evaporated under reduced pressure at 40°, and the residue was dissolved in the minimum quantity of N-sulphuric acid. The methylated-gum acid was extracted with chloroform and on removal of the chloroform was obtained as a crisp, yellow solid {11 g.; $[\alpha]_D^{10}$ +82° (*c*, 1·0)} [Found: OMe, 31·8%; sulphated ash, 1·5%; equiv. (by titration), 483].

The methylated-gum acid (28 g. from three batches) was esterified by two treatments with methyl iodide and silver oxide, and the product {26·2 g.; $[\alpha]_D^{10}$ +94·5° (*c*, 0·55)} was isolated by removal of the solvent (Found: OMe, 40·6%; equiv., 420).

Fractionation of the Methylated Polysaccharide.—The methylated polysaccharide (20 g.) was heated under reflux with mixtures of light petroleum (b. p. 40—60°) and chloroform. The extracts were evaporated under reduced pressure and the following fractions obtained:

Fraction I [extracted with light petroleum (90 vols.)–chloroform (10 vols.)], 1·79 g.; $[\alpha]_D^{10}$ +46° (*c*, 0·5 in methanol) [Found: OMe, 40·5%; equiv. (by quantitative alkaline hydrolysis), 420].

Fraction II [extracted with light petroleum (80 vols.)–chloroform (20 vols.)], 4·32 g.; $[\alpha]_D^{10}$ +61° (*c*, 0·77 in methanol) (Found: OMe, 43·0%; equiv., 420).

Fraction III [extracted with light petroleum (75 vols.)–chloroform (25 vols.)], 11·41 g.; $[\alpha]_D^{10}$ +60° (*c*, 0·62 in methanol) (Found: OMe, 42·9%; equiv., 420).

Fraction IV (residue), 1·41 g.; $[\alpha]_D^{10}$ +64° (*c*, 0·98 in methanol) (Found: OMe, 38·0; sulphated ash, 0·2%; equiv., 358).

A small portion of each fraction was heated with 4% methanolic hydrogen chloride in a sealed tube for 24 hours, hydrolysed with 4% hydrochloric acid, and neutralised with Amberlite resin IR-4B. The methylated sugars were separated by partition chromatography on filter-paper strips (Hirst, Hough, and Jones, *J.*, 1949, 928). Each chromatogram, on development with ammoniacal silver nitrate, showed the same pattern, consisting of five spots at R_G 0·88, 0·715, 0·57, 0·44 (traces), and 0·30 (traces), corresponding to 2 : 3 : 4 : 6-tetramethyl galactose, 2 : 3 : 6-trimethyl galactose, 4-methyl rhamnose, 2 : 6-dimethyl galactose, and rhamnose, respectively.

Hydrolysis of the Methylated Polysaccharide.—Methylated *Sterculia* gum (fraction III; 4·5 g.) was dissolved in 4% methanolic hydrogen chloride (100 ml.), and the solution was heated in a sealed tube at 100° for 24 hours, cooled, and neutralised with a cold ethereal solution of diazomethane, and the solvents were evaporated. The residual syrup (5·5 g.) was dissolved in 4% hydrochloric acid, and the solution heated under reflux at 100° for 3 hours. It was neutralised with silver carbonate, and silver ions were removed from the filtrate by the passage of hydrogen sulphide, followed by filtration. The barium salts of the uronic acids were formed by the addition of barium carbonate to the solution, the excess of barium carbonate was removed by filtration, and the solution was evaporated to dryness. The residue (*A*) (5 g.) was extracted with boiling ether (4 portions; each 100 ml.), and the insoluble barium salts (2·8 g.) were collected and dried.

Analysis and Identification of the Methylated Neutral Reducing Sugars from the Residue (A).—The ether extracts were combined and evaporated to a syrup (2·07 g.). A portion (0·78 g.) of this was separated by partition chromatography on a column of cellulose into four fractions, each of which gave one spot only on the paper chromatogram at R_G 0·88, 0·71, 0·57, and 0·44, respectively. A mixture of light petroleum (b. p. 100—120°) (70%) and *n*-butanol (30%) was used as the mobile phase. After separation, the solvent was removed by distillation under reduced pressure, and the residues (from each fraction) were dissolved in water. Small precipitates (which gave a positive Molisch test and were probably a modified form of cellulose) were removed by filtration, and the solutions were evaporated, giving, respectively:

Fraction A (0·404 g.): 2 : 3 : 4 : 6-tetramethyl D-galactose and dimethyl L-rhamnoses, R_G, 0·88, $[\alpha]_D^{18}$ +67° (*c*, 1·0) (Found: OMe, 44·3%).

Fraction B (0·141 g.): 2 : 3 : 6-trimethyl D-galactose, R_G, 0·71, $[\alpha]_D^{18}$ +87° (*c*, 1·2) (Found: OMe, 38·9%. Calc. for $C_9H_{18}O_6$: OMe, 41·8%).

Fraction C (0·117 g.): methyl L-rhamnose, R_G, 0·57, $[\alpha]_D^{18}$ +30° (*c*, 1·06) (Found: OMe, 17·9%. Calc. for $C_8H_{12}O_5$: OMe, 17·4%).

Fraction D (0·024 g.): 2 : 6-dimethyl D-galactose, R_G, 0·44, $[\alpha]_D^{18}$ +68° (*c*, 0·80) (Found: OMe, 30·2%. Calc. for $C_8H_{16}O_6$: OMe, 29·8%).

Subsequently, further quantities of the methylated sugars were separated on the column of cellulose by this procedure.

Examination of the Fractions.—*Fraction* A. The methoxyl content and the R_G value indicated the presence of 60% of tetramethyl galactose and 40% of dimethyl rhamnose.

A portion (1·01 g.) was fractionally distilled giving:

Fraction *A*1 (0·09 g.): b. p. 145—150°/0·001 mm., $[\alpha]_D^{18}$ +79° (*c*, 1·7) (Found: OMe, 47%. Calc. for tetramethyl galactose: OMe, 52·3; for dimethyl rhamnose: OMe, 32·3%).

Fraction *A*2 (0·444 g.): b. p. 150—160°/0·001 mm., $[\alpha]_D^{18}$ +71° (*c*, 0·87) (Found: OMe, 45·8%).

Fraction *A*3 (0·14 g.): b. p. 160—170°/0·001 mm., $[\alpha]_D^{18}$ +68° (*c*, 0·59) (Found: OMe, 39·6%).

Fraction *A*4 (residue) (0·16 g.): $[\alpha]_D^{18}$ +52·5° (*c*, 0·70) (Found: OMe, 42·9%).

Fraction A1. A portion of this fraction (0·07 g.) was heated under reflux with aniline (0·04 g.) in alcohol (3 ml.) for 2 hours at 100°, and gave, on cooling, crystals of 2 : 3 : 4 : 6-tetramethyl D-galactose anilide. The crystals were filtered off and washed with a little alcohol; (0·03 g.) m. p. and mixed m. p. 194° (Found: OMe, 39·5. Calc. for $C_{16}H_{25}O_5N$: OMe, 39·9%).

Fraction A2. A portion (26·2 mg.) of this fraction was treated with sodium periodate. The equivalent of 1 mole of sodium periodate was consumed by 1048 g. of the fraction, corresponding to 18% of dimethyl rhamnose.

Fraction A3. A portion (23·8 mg.) was treated with sodium periodate; the equivalent of 1 mole of periodate was consumed by 794 g. of the fraction.

In view of the inability to separate dimethyl rhamnose in good yield by fractional distillation, fractions *A*2, *A*3, and *A*4 were combined (0·56 g.), and the constituents converted into their anilides. Attempts to obtain a dimethyl rhamnose anilide were unsuccessful, only 2 : 3 : 4 : 6-tetramethyl D-galactose anilide (0·3 g.; m. p. and mixed m. p. 194°) being isolated.

Fraction B. The methoxyl content (38·9%) and R_G value (0·71) suggested that this fraction consisted of 2 : 3 : 6-trimethyl galactose. A portion (0·043 g.) was dissolved in 4% methanolic hydrogen chloride (10 ml.), and the optical rotation of the solution was observed: $[\alpha]_D$ +41° (initial value), −19° (17 hours), −43° (44 hours), −48° (68 hours; constant value). These observations indicate that the sugar derivative possesses a free hydroxyl group on $C_{(4)}$, and the behaviour is typical of that of 2 : 3 : 6-trimethyl D-galactose.

A portion (0·092 g.) was oxidised with bromine water at room temperature for 48 hours. The bromine was removed by aeration, and the solution was neutralised by the addition of silver carbonate. The insoluble silver salts were filtered off and the silver was removed with hydrogen sulphide. The solution was evaporated under reduced pressure, whereupon crystals of 2 : 3 : 6-trimethyl γ-D-galactonolactone separated (0·065 g.). After recrystallisation from ether–light petroleum, the crystals had m. p. and mixed m. p. 98°, $[\alpha]_D^{18}$ −40° (*c*, 0·85) [Found: OMe, 42·6%; equiv. (by titration), 220. Calc. for $C_9H_{16}O_6$: OMe, 42·4%; equiv., 222].

Fraction C. A portion (0·095 g.) of this fraction was oxidised with bromine water at room temperature for 48 hours and the resulting acid was isolated. It was heated at 100° under reduced pressure for one hour, and the syrupy lactone (0·086 g.) was distilled in a high vacuum, giving 2(or 3)-*methyl*-γ-L-*rhamnonolactone*; $[\alpha]_D^{18\cdot5}$ −28° (initial value; *c*, 0·43) ⟶ −21° (16 hours) (Found: OMe, 18·1%; equiv., 180. $C_7H_{12}O_5$ requires OMe, 17·7%; equiv., 176) [compare: 4-methyl δ-L-rhamnonolactone, $[\alpha]_D$ −141° (initial value) (Gill, Hirst, and Jones, *J.*, 1939, 1469; 1946, 1025); δ-L-rhamnonolactone, $[\alpha]_D$ −98·4° (initial value) (Jackson and Hudson, *J. Amer. Chem. Soc.*, 1930, **52**, 1270); γ-L-rhamnonolactone, $[\alpha]_D$ −39·7° (initial value) (Jackson and Hudson, *loc. cit.*)].

The lactone (10·7 mg.) was slowly neutralised with 0·01N-sodium hydroxide, sodium periodate (0·5M.; 1 ml.) was added, and the acetaldehyde produced was determined. The equivalent of 1 mole of methyl rhamnonolactone gave 0·47 mole of acetaldehyde. Unfortunately, no authentic specimens of monomethyl rhamnoses were available and it was not possible to carry out blank experiments.

Fraction D. This crystallised when it was seeded with a specimen of 2 : 6-dimethyl D-galactose. The crystals were separated on a tile, recrystallised from acetone, and dried (P_2O_5) at 60° under reduced pressure; m. p. 128°, undepressed on admixture with an authentic specimen of 2 : 6-dimethyl D-galactose (Found: OMe, 30·2. Calc. for $C_8H_{16}O_6$: OMe, 29·8%). 2 : 6-Dimethyl D-galactose forms a monohydrate, m. p. 90°.

Attempts to Isolate Tagatose Derivatives from Methylated Sterculia *Gum.*—The methylated sugars from the residue (*A*) (0·81 g.) were oxidised with bromine water until the solution was non-reducing to alkaline hypoiodite. Excess of bromine was removed by aeration, and the solution was filtered before and after the passage of hydrogen sulphide, neutralised with barium carbonate, and evaporated. The barium salts contained no ether-soluble material and were non-reducing to both Fehling's solution and ammoniacal silver nitrate, indicating the absence of tagatose derivatives.

Analysis and Identification of the Barium Salts from (A).—A portion (1 g.) of the barium salts (Found: OMe, 9·7; Ba, 24·2. Calc. for barium methyl galacturonate: OMe, 11·2; Ba, 24·6%) was dissolved in water (25 ml.), the barium was removed by use of Amberlite resin IR-100, and the solution was evaporated to a syrup {0·64 g.; $[\alpha]_D$ +52° (*c*, 0·63) [Found: OMe, 17·6%; equiv. (by titration), 230. Calc. for $C_7H_{12}O_7$: OMe, 14·9%; equiv., 208]}. A small portion was examined on the paper chromatogram, using the top layer of a mixture of butanol (40%), acetic acid (10%), and water (50%) as the mobile phase. The paper chromatogram was sprayed with a solution of aniline trichloroacetate in ethanol and then heated. It showed an intense red spot at R_G 0·15, corresponding to 2-methyl galacturonic acid, and faint spots at R_G 0·08, R_G 0·19, and R_G 0·30.

The acid was oxidised with bromine water until it no longer reduced Fehling's solution, and the solution was then neutralised with silver carbonate. Hydrogen sulphide was passed into the filtered solution and the silver sulphide removed by filtration. The filtrate was evaporated under reduced

pressure to a syrup (0·48 g.) which was converted into the dimethyl ester by heating it with 4% methanolic hydrogen chloride for 5 hours. The syrupy product (0·49 g.) was isolated after neutralisation of the solution with silver carbonate, and was distilled in a high vacuum; the product (0·35 g.) had $[\alpha]_D^{18\cdot5}$ −7·7° (*c*, 0·84) [Found: OMe, 33·3%; equiv. (by alkaline hydrolysis), 130. Calc. for $C_9H_{16}O_8$: OMe, 36·9%; equiv., 128]. The ester was converted into an amide by the action of liquid ammonia at 0°. After three days fine needles separated, and were roughly dried on a tile and recrystallised from methanol; m. p. 207°. The melting point of an authentic specimen of 2-methyl mucic acid diamide was undepressed on admixture with the crystals (Found: OMe, 12·6. Calc. for $C_7H_{14}O_6N_2$: OMe, 14·2%).

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