536. *The Acidic Sugar Components of* Cochlospermum gossypium *Gum.*

By G. O. Aspinall, E. L. Hirst, and (Miss) Margaret J. Johnston.

Methylation studies indicate the presence in *Cochlospermum gossypium* gum of D-glucuronic acid residues as end-groups and of D-galacturonic acid residues in the inner chains of the molecular structure. Partial acid hydrolysis of the gum affords a complex mixture of acidic oligosaccharides, including 2-*O*-(D-galactopyranosyluronic acid)-L-rhamnose. The known structural features of the gum are compared with those of gums of the *Khaya* and *Sterculia* genera.

The gum exudate from the bark of *Cochlospermum gossypium*, which is often known as kutira gum,* is a partly acetylated acidic polysaccharide. Previous studies [1] showed the occurrence of residues of L-rhamnose, D-galactose, and D-galacturonic acid, and methylation established the modes of linkage of the neutral sugar residues, but the modes of linkage of the acidic sugar residues were indicated with much less certainty. Some further information on the nature of these acidic sugar units is now available.

The hydrolysate from the methylated polysaccharide was separated into neutral and acidic sugars. Chromatographic examination of the neutral sugars confirmed the presence of those methylated sugars which had been identified previously,[1] namely, 2,3,4,6-tetra- and 2,3,6-tri-*O*-methyl-D-galactose, and 2,3,4-tri-, 3,4-di-, and 3-*O*-methyl-L-rhamnose. The methylated acidic sugars were converted into methyl ester methyl glycosides, reduced with lithium aluminium hydride, and hydrolysed to give a syrupy mixture of neutral methylated sugars, which was fractionated by partition on cellulose. The two main products were 2,3,4-tri-*O*-methyl-D-glucose and 3-*O*-methyl-D-galactose; small amounts of 3,4-di-*O*-methyl-D-glucose, 2,3,6-tri-, 2,3- and 3,4-di-, and 2-*O*-methyl-D-galactose, and 3-*O*-methyl-L-rhamnose were also present. Although not all these sugars were

* The name "karaya gum," which was previously given to the exudate from *Cochlospermum gossypium*,[1] is properly applied to *Sterculia urens* gum with which it is sometimes confused.

[1] Hirst and Dunstan, *J.*, 1953, 2332.

characterised by the formation of crystalline derivatives, they were identified by optical rotation and by paper chromatography in various solvent systems, and of the products of demethylation and of periodate oxidation.[2] With the exception of 2,3,6-tri-*O*-methyl-D-galactose and 3-*O*-methyl-L-rhamnose, none of these was present amongst the neutral products formed on direct hydrolysis of the methylated gum, and it may be assumed that they arose from the reduction of the corresponding methyl ethers of hexuronic acids. These results show clearly that residues of both D-glucuronic and D-galacturonic acid are present in the gum, the former being present mainly as non-reducing end-groups and accounting for approximately a quarter of the acidic groups, and the latter being found in the inner chains of the polysaccharide, in part as branching points.

In the previous investigation [1] the unresolved mixture of acidic oligosaccharides formed on partial acid hydrolysis of the gum was examined by the methylation method. The demonstration that D-glucuronic acid is a second acidic sugar constituent of the gum casts some doubt on the interpretation of the earlier results when it was assumed that D-galacturonic acid was the sole constituent acid. In order to obtain further information on the location of the acidic sugar units in the polysaccharide our small remaining sample of the gum was hydrolysed with dilute mineral acid, and the acidic hydrolysis products were separated from neutral sugars by adsorption on an anion-exchange resin. Subsequent partition by chromatography on filter sheets gave four fractions.

The first acidic sugar fraction was identified by paper chromatography as galacturonic acid and, after reduction of the methyl ester methyl glycosides followed by hydrolysis, as galactose. The second fraction was characterised as 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose by conversion into the crystalline methyl glycoside pentamethyl ether. The third fraction, although apparently homogeneous on paper chromatograms, was probably a mixture of acidic oligosaccharides. Reduction of the methyl ester methyl glycosides followed by hydrolysis gave galactose, smaller amounts of rhamnose and glucose, and a trace of an unknown sugar. The mixture was methylated, the methylated acids were reduced with lithium aluminium hydride, and the products were hydrolysed. Paper chromatography and ionophoresis of the sugars and their derivatives showed the presence of 2,3,4-tri-*O*-methylglucose, 2,3,4- and 2,3,6-tri- and 2,4-di-*O*-methylgalactose, and 3,4-di-*O*-methylrhamnose. The identity of four of these sugars was supported by gas–liquid chromatography of the derived methyl glycosides.[3] These methylated sugars probably arose from the following structural units, D-G*p*Al••, D-Gal*p*A 1 • •, • • 3 D-Gal*p*A 1••, • • 4 D-Gal*p* 1••, and • • 2 L-Rha*p* 1••. These results are clearly open to several interpretations, but it is likely that the sequence of sugar units in the aldobiouronic acid, 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, occurs in a higher oligosaccharide and that contiguous hexuronic acid units are present in one of the components of the mixture. The fourth fraction contained galactose, rhamnose, glucuronic acid, and probably galacturonic acid residues, but was not examined in detail.

Cochlospermum gossypium gum is a polysaccharide of great complexity and, although no detailed structure can yet be advanced, certain of its structural features may be compared with those of gums of the *Khaya* [4] and *Sterculia* [5–7] genera. These gums all contain a high proportion of acidic units, with D-galactose, L-rhamnose, and D-galacturonic, and/or D-glucuronic acid as the constituent sugars. Residues of D-glucuronic acid (or its 4-methyl ether) are present mainly as end groups, whereas those of D-galacturonic acid have been found only in the inner chains of the gums. *C. gossypium* gum is

[2] Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.
[3] Bishop and Cooper, *Canad. J. Chem.*, 1960, **38**, 388.
[4] Aspinall, Hirst, and Matheson, *J.*, 1956, 989; Aspinall, Johnston, and Stephen, *J.*, 1960, 4918.
[5] Hirst, Hough, and Jones, *J.*, 1949, 3145; Hough and Jones, *J.*, 1950, 1199.
[6] Hirst, Percival, and Williams, *J.*, 1958, 1942.
[7] Aspinall and Nasir-ud-din, unpublished results.

similar to the *Khaya* gums [4] and to *Sterculia setigera* [5] and *S. urens* [7] gums in giving rise to the aldobiouronic acid, 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, on partial hydrolysis, but it differs from *Brachychiton diversifolium* (*Sterculia caudata*) gum which affords 2-*O*-(α-D-glucopyranosyluronic acid)-L-rhamnose.[6] These various gums also contain sugar residues which are involved in the same types of linkages, but it is already apparent that there are some differences in the nature of the units which provide the branching points in the polysaccharides. However, further structural comparisons cannot be made until more detailed sequences of sugar residues have been established.

Experimental

The sample of gum (Found: OMe, 0·0%) was that used in the previous investigation. Paper chromatography was carried out on Whatman Nos. 1, 3MM, and 17 papers with the following solvent systems (v/v): (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (C) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (D) benzene–ethanol–water (169 : 47 : 15, upper layer). Unless otherwise stated, R_G values of methylated sugars refer to the rate of movement relative to 2,3,4,6-tetra-*O*-methyl-D-glucose in solvent C. Demethylation of methylated sugars were performed by the method of Hough, Jones, and Wadman.[8] Paper ionophoresis was in borate buffer at pH 10. Unless otherwise stated, optical rotations were observed for water solutions at *ca.* 18°.

Partial Hydrolysis of the Gum and Examination of Acidic Fractions.—The powdered gum (2·7 g.) was swollen in water (50 ml.) for 2 days, 2N-sulphuric acid (50 ml.) was added, and the suspension was warmed to give a clear solution, filtered to remove mechanical impurities, and heated at 100° for 12 hr. The cooled solution was neutralised with barium hydroxide and barium carbonate, filtered, treated with Amberlite resin IR-120(H^+) to remove barium ions, and concentrated to a syrup (1·47 g.) which was poured on a column of Amberlite resin CG-45 (formate form). Neutral sugars were eluted with carbon dioxide-free water, and acidic sugars (0·64 g.) were eluted with 15% formic acid and separated on filter sheets by means of solvent B into four fractions.

Fraction A (22 mg.) had $R_{\text{galacturonic acid}}$ 1·0 in solvent B and after reduction of the methyl ester methyl glycosides followed by hydrolysis gave galactose.

Fraction B (165 mg.) had $R_{\text{galacturonic acid}}$ 0·76 in solvent B. Reduction of the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave galactose and rhamnose. The aldobiouronic acid was characterised as 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose by conversion into the methyl glycoside pentamethyl ether dihydrate,[9] which was identified by m. p. and mixed m. p. 67—69°, $[\alpha]_D$ +92° (*c* 0·49 in $CHCl_3$), and *X*-ray powder photograph.

Fraction C (191 mg.), $R_{\text{galacturonic acid}}$ 0·14, after reduction of the methyl ester methyl glycosides and hydrolysis, gave galactose, smaller amounts of glucose and rhamnose, and a trace of an unknown sugar, R_{rhamnose} 1·03 in solvent A. The syrup (180 mg.) was methylated with two additions of methyl sulphate (11 ml.) and 30% aqueous sodium hydroxide (25 ml.), and methylated acids (86 mg.) were isolated by extraction with chloroform. The methylated acids were reduced with lithium aluminium hydride in tetrahydrofuran to give methylated neutral oligosaccharides (76 mg.) which were hydrolysed with N-hydrochloric acid at 100° for 4 hr. The major portion (45 mg.) of the resulting mixture of methylated sugars was separated on filter sheets with solvent C, to give three fractions having R_G 0·89, 0·73, and 0·47. Fraction *a* was shown by chromatography in solvent D and by ionophoresis to contain 2,3,4-tri-*O*-methylglucose and 3,4-di-*O*-methylrhamnose. Fraction *b* gave only galactose on demethylation, and chromatography in solvent A showed 2,3,4- and 2,3,6-tri-*O*-methylgalactose. Fraction *c* gave galactose on demethylation and was unattacked by periodate. Periodate oxidation of the derived glycitol (from borohydride reduction) gave a di-*O*-methylpentose chromatographically indistinguishable from the product similarly derived from 2,4-di-*O*-methyl-D-galactose. The remainder of the mixture of sugars was converted into methyl glycosides, which were examined by Dr. C. T. Bishop by gas–liquid partition chromatography.[3] A Pye argon chromatograph was used with 4 ft columns of (i) 10% butane-1,4-diol–succinate polyester on Chromosorb W, and

[8] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[9] Aspinall and Fanshawe, *J.*, 1961, 4215.

(ii) 10% of *m*-di-(*m*-phenoxyphenoxy)benzene on Chromosorb W, at 180° at gas flow rates of 100 ml./min. As indicated in the annexed Table, the mixture contained substances having the retention times of methyl glycosides of 3,4-di-*O*-methyl-L-rhamnose, 2,3,4-tri-*O*-methyl-D-glucose, and 2,3,4- and 2,3,6-tri-*O*-methyl-D-galactose on both liquid phases. When the mixture was examined on column (ii) at 200°, two further components were shown to be present, whose retention times were consistent with those of the methyl glycosides of a di-*O*-methylhexose.

Relative retention times of methyl glycosides.

	Column (i)		Column (ii)	
Methyl ether	Authentic sample	Mixture	Authentic sample	Mixture
3,4-Di-*O*-methyl-L-rhamnose	1·00	1·00	1·00	1·00
	1·83	1·82	1·85	1·83
2,3,4-Tri-*O*-methyl-D-glucose	2·63	2·64	2·32	2·34
	3·64	3·63	3·26	3·26
2,3,6-Tri-*O*-methyl-D-galactose	3·20	3·21	2·99	2·99
			4·83	4·78 (?)
2,3,4-Tri-*O*-methyl-D-galactose	4·59	4·59	4·92	4·95 (?)
	7·20	7·21	5·50	5·53

Fraction D (31 mg.), $R_{\text{galacturonic acid}}$ 0·05 in solvent B, was shown to contain three components by chromatography in ethyl acetete–acetic acid–formic acid–water (18 : 8 : 3 : 9). Hydrolysis gave galactose, rhamnose, and acidic sugars. Reduction of the methyl ester methyl glycosides followed by hydrolysis gave galactose, glucose, and rhamnose.

Examination of Acidic Sugars from Hydrolysis of the Methylated Gum.—The methylated gum, $[\alpha]_D$ +58° (*c* 0·54 in $CHCl_3$), was prepared as described previously [1] (Found: OMe, 37·6%, not raised on further methylation). The methylated gum (5·05 g.) was hydrolysed by 90% formic acid (200 ml.) at 100° for 25 hr. Formic acid was removed under reduced pressure, and the resulting syrup was heated with 0·5N-sulphuric acid at 100° for 2 hr. to hydrolyse formyl esters. The cooled solution was neutralised with barium carbonate, filtered, and concentrated to a syrup which was placed on a cellulose column (35 × 600 mm.). Neutral methylated sugars were eluted with butan-1-ol half-saturated with water, and chromatography showed the presence of 2,3,4-tri-*O*-methylrhamnose (R_G 1·01), 2,3,4,6-tetra-*O*-methylgalactose (R_G 0·88), 3,4-di-*O*-methylrhamnose (R_G 0·84), 2,3,6-tri-*O*-methylgalactose (R_G 0·75), 3-*O*-methylrhamnose (R_G 0·60), and a trace of rhamnose (R_G 0·32). Elution with water gave the barium salts of acidic sugars, which were treated with Amberlite resin IR-120(H^+) (to remove barium ions) and concentrated to a syrup (1·8 g.). The mixture of acidic sugars was refluxed with methanolic 2·6% hydrogen chloride for 6 hr., and lithium aluminium hydride (2 g.) in tetrahydrofuran (30 ml.) was added to the resulting methyl ester methyl glycosides (1·7 g.) in boiling tetrahydrofuran (30 ml.) during 1 hr. Heating was continued for 1 hr., the excess of hydride was destroyed by the addition of water, the resulting precipitate of metal hydroxides was dried, the filtrate was taken to dryness, and both residues were extracted with chloroform and acetone to give a syrupy mixture (1·0 g.) of sugars. This mixture was separated on cellulose (25 × 450 mm.) with light petroleum (b. p. 100—120°)–butan-1-ol (1 : 1), saturated with water, and butan-1-ol, half-saturated with water, as eluents, into seven fractions.

Fraction 1. The syrup (196 mg.), R_G 0·85 and $[\alpha]_D$ +66° (*c* 1·84), was chromatographically indistinguishable from 2,3,4-tri-*O*-methyl-D-glucose. Demethylation gave glucose. Periodate oxidation of the derived glycitol (from borohydride reduction) gave 2,3,4-tri-*O*-methylxylose. Attempts to prepare the crystalline aniline derivative were unsuccessful.

Fraction 2. The mixture (73 mg.) of sugars, R_G 0·78, 0·71, and 0·63, was fractionated on filter sheets with solvent D. Fraction 2*a* (24 mg.), R_G 0·78, was chromatographically indistinguishable from 2,3,6-tri-*O*-methyl-D-galactose in solvents A and C, and gave galactose on demethylation. Fraction 2*b* (12 mg.) R_G 0·71 gave galactose on demethylation. Fraction 2*c* (11 mg.), R_G 0·63, gave glucose on demethylation. Periodate oxidation [2] gave a sugar chromatographically identical with 2,3-di-*O*-methyl-L-arabinose, which was similarly formed from 3,4-di-*O*-methyl-D-mannose. It is concluded that the sugar is 3,4-di-*O*-methyl-D-glucose.

Fraction 3. Ionophoresis of the syrup (12 mg.), R_G 0·59 and $[\alpha]_D$ +57° (*c* 1·2), showed the

presence of 3-*O*-methylrhamnose (M_G 0·41) as the main component together with other components (M_G 0·12 and 0·59). The presence of 3-*O*-methylrhamnose was further indicated by examination of the periodate oxidation products.

Fraction 4. The sugar (14 mg.), R_G 0·50, was chromatographically and ionophoretically indistinguishable from 2,3-di-*O*-methyl-D-galactose. The presence of this sugar was further indicated by chromatographic examination of the periodate oxidation products from the derived glycitol (from borohydride reduction) and by the formation of galactose on demethylation.

Fraction 5. The major component of the mixture (23 mg.), R_G *ca.* 0·42, was shown to be 3,4-di-*O*-methylgalactose by chromatography and ionophoresis of the sugar, and by chromatography of the periodate oxidation products of the sugar and of the derived glycitol (from borohydride reduction). Demethylation of the mixture gave only galactose.

Fraction 6. The crystalline sugar (60 mg.), R_G 0·31 and $[\alpha]_D$ +68° (equil.) (*c* 0·34), had m. p. and mixed m. p. (with 2-*O*-methyl-D-galactose) 143—147°. Periodate oxidation of the sugar [2] gave methoxymalondialdehyde, and the derived methyl glycosides consumed 1 mol. of periodate.

Fraction 7. Chromatography of the sugar (133 mg.), R_G 0·24 and $[\alpha]_D$ +86° (*c* 0·44), and of its periodate oxidation products [2] indicated the presence of 3-*O*-methylgalactose. Demethylation gave galactose. The derived methyl glycosides did not reduce periodate. After recrystallisation from ethanol-acetone the sugar had m. p. and mixed m. p. (with 3-*O*-methyl-D-galactose) 140—142°.

The authors thank the Carnegie Trust for the Universities of Scotland for the award of a scholarship (to M. J. J.), and the Distillers Company Limited for a grant. They are grateful to Dr. C. T. Bishop for vapour-phase chromatography and its interpretation.

DEPARTMENT OF CHEMISTRY, UNIVERSITY OF EDINBURGH. [*Received, December 22nd,* 1961.]