798. *Plant Gums of the Genus* Sterculia. *Part III.*[1] Sterculia setigera *and* Cochlospermum gossypium *Gums*

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Partial hydrolysis of *Sterculia setigera* and *Cochlospermum gossypium* gums furnishes similar mixtures of acidic oligosaccharides, including 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, 4-*O*-(β-D-galactopyranosyluronic acid)-D-galactose, 3-*O*-(β-D-glucopyranosyluronic acid)-D-galacturonic acid, and *O*-(β-D-glucopyranosyluronic acid)-(1→3)-*O*-(α-D-galactopyranosyluronic acid)-(1→2)-L-rhamnose. The known structural features of the gums are compared with those of other *Sterculia* gums in the light of these results and of a re-examination of the cleavage products from the methylated polysaccharides.

Earlier studies of *Sterculia setigera* [2] and *Cochlospermum gossypium* [3] gums indicated that the two highly branched polysaccharides, which contained residues of D-galacturonic acid, D-galactose, and L-rhamnose, had several structural features in common. Partial hydrolysis of the two gums furnished similar mixtures of acidic oligosaccharides, and, although the individual components of the mixtures were not separated, some indication of their nature was obtained by methylation of the mixtures. In the case of *C. gossypium* gum, however, later work [4] established the presence of D-glucuronic acid in addition to D-galacturonic acid residues. A further examination of the partial hydrolysis products led to characterisation of 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, but no discrete oligosaccharides containing D-glucuronic acid residues could be isolated although the presence of at least one such oligosaccharide in a mixture was demonstrated. Since recent studies of *Sterculia urens* [5] and *S. caudata* [1,6] gums have shown that the polysaccharide components of these gums also contain residues of D-glucuronic and D-galacturonic acids, the partial hydrolysis products of *S. setigera* gum have now been re-examined together with those from *C. gossypium* gum.

Cochlospermum gossypium gum has sometimes been erroneously referred to as " karaya gum," a term properly applied to *Sterculia urens*. In view of possible doubts as to the origin of the commercial sample of gum used in the previous studies,[2,4] gum from a botanically authenticated source was used in the present investigation, and we are grateful to Mr. A. G. Kenyon of the Tropical Products Institute for arranging for the supply of this material and an authentic sample of *S. urens* gum, and also to Professor J. K. N.

[1] Part II, G. O. Aspinall and R. N. Fraser, preceding Paper.
[2] E. L. Hirst, L. Hough, and J. K. N. Jones, *J.*, 1949, 3145; L. Hough and J. K. N. Jones, *J.*, 1950, 1199.
[3] E. L. Hirst and S. Dunstan, *J.*, 1953, 2332.
[4] G. O. Aspinall, E. L. Hirst, and M. J. Johnston, *J.*, 1962, 2785.
[5] G. O. Aspinall and Nasir-ud-din, *J.*, 1965, 2710.
[6] E. L. Hirst, E. E. Percival, and R. S. Williams, *J.*, 1958, 1942.

Jones for generously providing us with some of the batch of *Sterculia setigera* gum on which previous studies were performed.

The polysaccharides from *Sterculia urens*, *S. setigera*, and *Cochlospermum gossypium* gums were obtained after deacetylation of the native gums with aqueous ammonia. The polysaccharides were methylated and the cleavage products from the methylated polysaccharides were examined by paper chromatography of the sugars and gas chromatography of the methyl glycosides. In addition, the mixtures of acidic sugars formed on hydrolysis of the methylated polysaccharides were treated with methanolic hydrogen chloride, reduced with sodium borohydride, and hydrolysed to give mixtures of sugars which were examined by appropriate chromatographic procedures. Neutral methylated hexoses which were detected only after reduction with borohydride were presumed to have arisen from the corresponding methylated hexuronic acids. Table 1 summarises the methylated sugars which have been characterised as cleavage products from the methylated polysaccharides from the three Sterculia gums (*S. urens*, *S. caudata*, and *S. setigera*) and *Cochlospermum gossypium* gum in the present and previous investigations.

The results in Table 1 show clearly that the various gums contain the same structural units. In the case of *Sterculia urens* gum no differences in structural units were observed between the commercial sample previously examined [5] and the present botanically authenticated specimen. Likewise, the botanically authenticated sample of *Cochlospermum gossypium* gum was similar in most respects to the earlier sample of commercial origin.[3,4] The polysaccharides from the various gums probably differ to a relatively small extent in the proportions of the constituent sugars, but methylation studies have failed to reveal any important qualitative differences in the nature and mode linkage of these units.

TABLE 1

Methylated sugar residues in methylated gums

Gum:	*Sterculia urens*		*Sterculia caudata*		*Sterculia setigera*		*Cochlospermum gossypium*		
Sample:	*a*	*b*	*b*		*b*		*a*	*b*	
Investigation:	*c* [5]	*d*	*c* [6]	*e* [1]	*c* [2]	*d*	*c* [3,4]	*d*	Approx.
Methylated sugar [f]									rel. propn. [g]
Me_4Gal	A	C	A	C	A	C	A	C	+++
2,3,6-Me_3Gal	A	C	A	C	A	C	A	C	+++
2,6-Me_2Gal	A	C		C		C	A	C	+
2,3,4-Me_3Rha	A	C		C		C	A	C	++
3,4-Me_2Rha	A	C	A	C	B	C	A	C	++
3-MeRha	A	C	A	C	B	C	A	C	++
2,3,4-Me_3GA	A	C	A	C		C	A	C	++
3,4-Me_2GA							B		trace
2,3,4-Me_3GalA		C		C		C		C	trace
2,3-Me_2GalA	A	C		C		C	B	C	+
2-Me GalA	A	C		C	A	C	A	C	++
3-MeGalA	A	C		C		C	A	C	+++

a, Commercial sample. *b*, Botanically authenticated sample. *c*, Previous work. *d*, Present work. *e*, Preceding Paper. *f*, Characterisation of methylated sugar: A, by formation of crystalline derivative; B, by paper chromatography of separated sugar component; C, by paper chromatography of sugar and/or gas chromatography of methyl glycosides in admixture with other sugar components. *g*, Semi-quantitative estimate of relative proportions based on isolated yields, relative intensities of spots on paper chromatograms, or relative peak intensities on gas chromatograms.

Further evidence for the structural similarities between the polysaccharides from these gums has been obtained by a re-examination of the acidic oligosaccharides formed on partial acid hydrolysis of the polysaccharides from *Sterculia setigera* and *Cochlospermum gossypium* gums. As in the preceding Paper [1] the complex mixtures of oligosaccharides were separated by ion-exchange chromatography on diethylaminoethyl-Sephadex followed as necessary by filter-sheet chromatography. Where sufficient quantities were obtained the oligosaccharides were characterised by the formation of crystalline derivatives, most frequently of the hydrolysis products of the methylated derivatives. Oligosaccharides,

which were isolated only in small amounts, were characterised by appropriate chromatographic procedures and by comparison of their infrared spectra with those of the corresponding oligosaccharides from *Sterculia caudata* gum.[1] 2-*O*-(α-D-Galactopyranosyluronic acid)-L-rhamnose, 4-*O*-(β-D-galactopyranosyluronic acid)-D-galactose, 3-*O*-(β-D-glucopyranosyluronic acid)-D-galacturonic acid, and *O*-(β-D-glucopyranosyluronic aid)-(1→3)-*O*-(α-D-galactopyranosyluronic acid)-(1→2)-L-rhamnose were identified as partial hydrolysis products from both gums. In addition, the partially acetylated oligosaccharide, 2-*O*-acetyl-4-*O*-(β-D-galactopyranosyluronic acid)-D-galactose, was obtained from both polysaccharides. It is evident again that the procedure used for the isolation of the polysaccharides which involves deacetylation of the native gums with aqueous ammonia does not result in complete deacylation. An additional trisaccharide, which was probably *O*-(D-galactopyranosyluronic acid)-(1→4)-*O*-(D-galactopyranosyluronic acid)-(1→2)-L-rhamnose, was isolated from *Sterculia setigera* gum, and chromatographic evidence was obtained for its presence amongst the partial hydrolysis products from *Cochlospermum gossypium* gum.

It is now apparent that the gums from the three *Sterculia* species (*S. urens*, *S. caudata*, and *S. setigera*) and the gum from *Cochlospermum gossypium* contain not only the same structural units but also that many of the same sequences of sugar residues are present in the four polysaccharides. Table 2 indicates the acidic oligosaccharides which have now been characterised as partial hydrolysis products of the gums. The various partial structures, which have been proposed for *Sterculia urens* [5] and *S. caudata* [1] gums, may now be extended to *S. setigera* and *C. gossypium* gums. In order to obtain further structural information on these gums it will be necessary to isolate, as products of partial degradation, fragments containing new sequences of sugar residues, and experiments to modify the relative rates of cleavage of the different glycosidic linkages are in progress.

TABLE 2

Acidic oligosaccharides from partial hydrolysis

Gum:	*Sterculia urens*	*Sterculia caudata*	*Sterculia setigera*	*Cochlospermum gossypium*
Oligosaccharide				
GalA 1 → 2 Rha	+	+	+	+
GalA 1 → 4 Gal.....	+	+	+	+
GA 1 → 3 GalA		+	+	+
GA 1 → 3 GalA 1 → 2 Rha...	+	+	+	+
GalA 1 → 4 GalA 1 → 2 Rha	?	+	+	?

EXPERIMENTAL

The general experimental procedures were as described in Part II.[1]

Isolation of Polysaccharides from Gums.—Finely powdered *Cochlospermum gossypium* gum (50 g.) was deacetylated with aqueous ammonia, as described for *Sterculia caudata*,[1] and afforded gum acid (34 g.), $[\alpha]_D$ +66° (*c* 0·49 in H_2O containing 1% of ammonia), uronic anhydride (by decarboxylation) 40·4%. Likewise, *Sterculia urens* gum furnished gum acid, $[\alpha]_D$ +59° (*c* 0·56 in 1% NaOH), uronic anhydride, 4V%. *Sterculia setigera* gum [2] was similarly deacetylated. Chromatography of the hydrolysates from each of the gums showed the presence of galactose, rhamnose, galacturonic and glucuronic acids, and complex mitures of acidic oligosaccharides.

Partial Hydrolysis of Gum Acids and Separation of Acidic Sugars.—Gum acid (10 g.) from *Cochlospermum gossypium* gum was heated in N-sulphuric acid (500 ml.) on a boiling-water bath for 7 hr. and afforded a syrupy hydrolysate (6·81 g.). The mixture of sugars was fractionated by column chromatography on diethylaminoethyl-Sephadex as described in Part II,[1] followed as required by filter-sheet chromatography in solvent B, to give neutral sugars (rhamnose, galactose, and *O*-acetylgalactose, 1·177 g.), galacturonic acid (759 mg.), oligosaccharide I (231 mg.), R_{GalA} 0·79 and $[\alpha]_D$ +93° (*c* 2·31 in H_2O), oligosaccharide II (104 mg.), R_{GalA} 0·39 and $[\alpha]_D$ +70° (*c* 1·9 in H_2O), oligosaccharide III (394 mg.), R_{GalA} 0·24 and $[\alpha]_D$ +120° (*c* 1·97 in H_2O), oligosaccharide IV (61 mg.), R_{GalA} 0·29 and $[\alpha]_D$ +42° (*c* 3·05 in H_2O), and oligosaccharide V (300 mg.), R_{GalA} 0·20 and $[\alpha]_D$ +75° (*c* 3·00 in H_2O). In addition,

a sugar with the chromatographic mobility of acidic oligosaccharide VI, R_{GalA} 0·18, from *Sterculia caudata* gum [1] was present but was not obtained in suitable form for detailed study.

Gum acid (2 g.) from *Sterculia setigera* gum was hydrolysed similarly to give a syrupy hydrolysate (1·296 g.). Fractionation of the syrup gave neutral sugars (183 mg.), galacturonic acid (245 mg.), oligsaccharide I (71 mg.), R_{GalA} 0·80 and $[\alpha]_D$ +94° (*c* 1·42 in H_2O), oligosaccharide II (31 mg.), R_{Gal} 0·39 and $[\alpha]_D$ +74° (*c* 1·55 in H_2O), oligosaccharide III (79 mg.), R_{GalA} 0·24 and $[\alpha]_D$ +122° (*c* 1·39 in H_2O), +122° ⟶ +76° (4 hr., equil.) (*c* 1·39 in H_2O containing 1% of ammonia), oligosaccharide IV (34 mg.), R_{GalA} 0·27 and $[\alpha]_D$ +42° (*c* 1·7 in H_2O), oligosaccharide V (64 mg.), R_{GalA} 0·24 and $[\alpha]_D$ +80° (*c* 1·85 in H_2O), and oligosaccharide VI (15 mg.), R_{GalA} 0·17 and $[\alpha]_D$ +73° (*c* 1·5 in H_2O).

Examination of Acidic Oligosaccharides.—Acidic oligosaccharides from both gums were examined by the following reaction sequences: (1) hydrolysis and paper chromatography of the hydrolysate; (2) reduction with sodium borohydride, hydrolysis, and paper chromatography of the hydrolysate; (3) conversion into the methyl ester methyl glycosides with methanolic hydrogen chloride, reduction with sodium borohydride, hydrolysis, and paper chromatography of the hydrolysate; and (4) methylation with methyl sulphate and sodium hydroxide, and methyl iodide and silver oxide, reduction with lithium aluminium hydride, methanolysis, and gas chromatography of the resulting methyl glycosides on column b. The results are summarised in Table 3. In addition, oligosaccharides I, III, IV, and V from both gums, and oligosaccharide II from *Cochlospermum gossypium* gum gave infrared spectra which were identical with those of the corresponding acidic oligosaccharides from *Sterculia caudata* gum.[1]

TABLE 3

Examination of acidic oligosaccharides

Acidic oligosaccharide	Reaction sequence	Products
I	1	Galacturonic acid, rhamnose
	2	Galacturonic acid, rhamnitol
	3	Galactose, rhamnose
II	1	Galacturonic acid, galactose
	2	Galacturonic acid, galactitol
	3	Galactose
	4	2,3,4- and 2,3,6-Me_3galactose
III	1	Galacturonic acid, galactose, *O*-acetylgalactose
	2	Galacturonic acid, galactitol
	3	Galactose
	4	2,3,4- and 2,3,6-Me_3galactose
IV	1	Glucuronic acid, galacturonic acid
	2	Glucuronic acid, galactonic acid
	3	Glucose, galactose
	4	2,3,4-Me_3glucose, 2,4-Me_2galactose
V	1	Glucuronic acid, galacturonic acid, rhamnose
	2	Glucuronic acid, galacturonic acid, rhamnitol
	3	Glucose, galactose, rhamnose
	4	2,3,4-Me_3glucose, 2,4-Me_2galactose, 3,4-Me_2rhamnose
VI	1	Galacturonic acid, rhamnose (molar ratio, 2 : 1)
	2	Galacturonic acid, rhamnitol
	3	Galactose, rhamnose

Oligosaccharide I. The aldobiouronic acid from both gums was characterised as 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose by conversion into the methyl glycoside pentamethyl ether dihydrate,[7] which was identified by m. p. 67—68° and mixed m. p. 67°, and by *X*-ray powder photograph.

Oligosaccharide III. The oligosaccharide (200 mg.) from *Cochlospermum gossypium* gum was saponified with 4N-sodium hydroxide (10 ml.) on a boiling-water bath for 30 min. The reaction mixture was acidified and distilled, to give acetic acid which was characterised by conversion into 4-nitrobenzyl acetate, m. p. and mixed m. p. 77°.

Oligosaccharide V. The acidic trisaccharide (100 mg.) from *Cochlosperumum gossypium* gum was successively methylated to give methylated acidic trisaccharide, reduced with lithium aluminium hydride to give methylated trisaccharide, and hydrolysed to give a mixture (54 mg.)

[7] G. O. Aspinall and R. S. Fanshawe, *J.*, 1961, 4215.

of sugars. The mixture was separated on filter sheets using solvent D, to give 2,4-di-*O*-methyl-D-galactose (14 mg.), $[\alpha]_D$ +90° (*c* 1·4 in H_2O), which crystallised as the monohydrate, m. p. 99° and mixed m. p. 99—100°, and a mixture (27 mg.) of sugars which was further separated by ionophoresis in borate buffer to give 3,4-di-*O*-methyl-L-rhamnose (10 mg.), $[\alpha]_D$ +23° (*c* 1·0 in H_2O), m. p. 96° and mixed m. p. 95—96°, and 2,3,4-tri-*O*-methyl-D-glucose (11 mg.), $[\alpha]_D$ +66° (*c* 1·1 in H_2O), which was characterised as the aniline derivative, m. p. 135° and mixed m. p. 134—135°.

Methylation of Gum Acids,—Samples (*ca.* 1·5 g.) of gum acids were stirred in suspension in ethereal diazomethane, and the resulting polysaccharide methyl esters were methylated with methyl sulphate and barium hydroxide in dimethyl sulphoxide and *NN*-dimethylformamide as described by Kuhn and Trischmann.[8] Further methylations with methyl iodide and silver oxide furnished (i) methylated *Cochlospermum gossypium* gum, $[\alpha]_D$ +43° (*c* 1·64 in $CHCl_3$) (Found: OMe, 409%), methylated *Sterculia urens* gum, $[\alpha]_D$ +68° (*c* 1·24 in $CHCl_3$) (Found: OMe, 41·9%), and methylated *Sterculia setigera* gum, $[\alpha]_D$ +60° (*c* 1·26 in $CHCl_3$) (Found: OMe, 43·9%).

Samples of the methylated polysaccharides were heated with methanolic hydrogen chloride, and, in each case, examination of the cleavage products by gas chromatography on columns a and b indicated the presence of methyl glycosides of the following sugars (approximate relative proportions in parenthesis): 2,3,4- (+ or ++), 3,4-di- (++), and 3-*O*-methylrhamnose (++), 2,3,4,6-tetra- (+++) and 2,3,6-tri-*O*-methylgalactose (++), 2,3,4-tri-*O*-methylglucuronic acid (++), 2,3,4-tri- (trace or +), and 2,3-di-*O*-methylgalacturonic acid (+).

Samples (0·2 g.) of the methylated polysaccharides were refluxed with methanolic 4% hydrogen chloride (10 ml.) for 18 hr. The neutralised solutions were concentrated, heated with saturated barium hydroxide (10 ml.) at 60° for 2 hr., and passed through Amberilite resin IR-120(H) to remove barium ions. The concentrated solutions were adsorbed on columns (5 × 1 cm.) of diethylaminoethyl-Sephadex (formate form). Elution with water furnished neutral methyl glycosides, and elution with aqueous 1% formic acid gave acidic methyl glycosides. The neutral methyl glycosides were hydrolysed, and paper chromatography of the resulting mixtures of sugars in solvents A, D, and E showed the above-mentioned neutral sugars together with traces of 2,6-di-*O*-methylgalactose and rhamnose. The acidic methyl glycosides were heated with methanolic hydrogen chloride, reduced with sodium borohydride, and hydrolysed, and paper chromatography of the resulting mixtures of sugars showed 3,4-di- (+) and 3-*O*-methylrhamnose (+), 2,3,4-tri- (trace or +), 2,3-di- (+), 2- (++), and 3-*O*-methylgalactose (++), and 2,3,4-tri-*O*-methylglucose (+++).

The authors thank Professor Sir Edmund Hirst, F.R.S., for his interest and advice, the Cotton, Silk, and Man-Made Fibres Research Association for the award of a Shirley Fellowship (to R. N. F.), and the Carnegie Trust for the Universities of Scotland for the award of a vacation scholarship (to G. R. S.).

Department of Chemistry, The University,
West Mains Road, Edinburgh 9. [*Received, March 3rd*, 1965.]

[8] R. Kuhn and H. Trischmann, *Chem. Ber.*, 1963, **93**, 284.