487. Combretum leonense *Gum. Part I. Partial Hydrolysis of the Gum*

By G. O. Aspinall and V. P. Bhavanandan

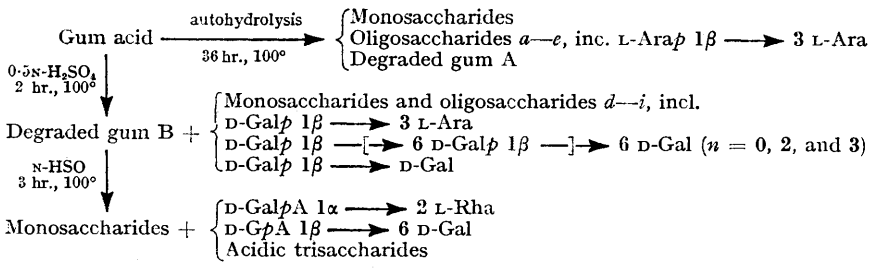
Graded acid hydrolysis of *Combretum leonense* gum, which was carried out in three stages, furnishes a complex mixture of neutral and acidic oligosaccharides, amongst which the following have been characterised: 3-*O*-β-L-arabinopyranosyl-L-arabinose, 3-*O*-β-D-galactopyranosyl-L-arabinose, 6-*O*-β D-galactopyranosyl-D-galactose, and the polymer-homologous trisaccharide 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, and 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose. The recognised structural features of the gum are compared with those of other gums.

A sample of *Combretum leonense* gum of Nigerian origin was kindly placed at our disposal by Professor R. J. McIlroy. The structural examination of this gum extends previous studies in this laboratory on the gum exudates of trees of the *Combretaceae* family, namely, those from *Anogeissus latifolia* (gum ghatti) [1] and *Anogeissus schimperi*.[2] Anderson, Hirst, and King [3] showed that the neutral sugar constituents of the gum, namely, arabinose, galactose, and rhamnose, are present in the approximate ratio of 9 : 10 : 1, but variations were noted in the uronic anhydride content (*ca.* 15—20%) of the polysaccharide which was isolated from different nodules of the gum. In this Paper the characterisation of some partial hydrolysis products of the gum is reported. In the following Paper [4] further structural studies are described and the nature of the polysaccharide heterogeneity in the gum is discussed.

The gum acid, which contained 15·2% of uronic anhydride, was prepared by precipitation of an acidified solution of the gum. In common with many other acidic polysaccharides from plant gums [5] this polysaccharide contained a variety of glycosidic linkages which were cleaved on acid hydrolysis at markedly different rates. Accordingly, graded hydrolysis was carried out in three stages as indicated on the flow sheet, degraded gums A and B being separated from the soluble sugars after the first and second stages. At each stage the various oligosaccharides were fractionated by adsorption and/or partition chromatography.

Autohydrolysis of the gum acid resulted in the cleavage of most of the arabinose and of a small proportion of the galactose residues, leaving degraded gum A composed largely of galactose, rhamnose, and hexuronic acid residues. At least five oligosaccharides were recognised amongst the soluble sugars. Oligosaccharide *a* was an arabinobiose of unknown structure. Oligosaccharide *b* was characterised as 3-*O*-β-L-arabinopyranosyl-L-arabinose by formation of its crystalline phenylosazone. Furthermore, the methanolysis products from the methylated disaccharide were shown by gas chromatography [6,7] to have the retention times of the methyl glycosides of 2,3,4-tri- and 2,4-di-*O*-methyl-L-arabinose, and, in trace amount, of 2,5-di-*O*-methyl-L-arabinose. From the mixture of sugars formed on hydrolysis of the methyl glycosides, 2,4-di-*O*-methyl-L-arabinose was separated and characterised. Oligosaccharide *c*, which was chromatographically indistinguishable from 3-*O*-β-D-galactopyranosyl-L-arabinose, was a mixture of disaccharides, the main component being an arabinosylgalactose since hydrolysis of the derived glycitols gave mainly arabinose and galactitol with small amounts of galactose and arabitol. Oligosaccharide *d*

[1] G. O. Aspinall, E. L. Hirst, and A. Wickstrøm, *J.*, 1955, 1160; G. O. Aspinall, B. J. Auret, and E. L. Hirst, *J.*, 1958, 221, 4408.
[2] G. O. Aspinall and T. B. Christensen, *J.*, 1961, 3461.
[3] D. M. W. Anderson, E. L. Hirst, and N. J. King, *Talanta*, 1959, **3**, 118.
[4] G. O. Aspinall and V. P. Bhavanandan, Part II, following Paper.
[5] F. Smith and R. Montgomery, " Chemistry of Plant Gums and Mucilages," Reinhold, New York, 1959.
[6] C. T. Bishop and F. P. Cooper, *Canad. J. Chem.*, 1960, **38**, 388.
[7] G. O. Aspinall, *J.*, 1963, 1676.



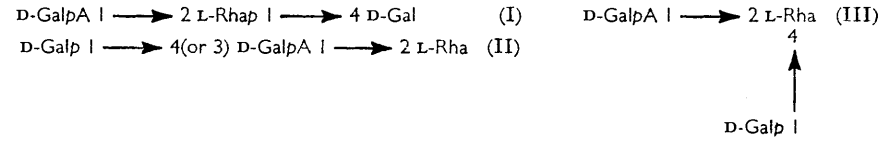
Scheme showing graded acid hydrolysis

was chromatographically indistinguishable from 4-*O*-β-D-galactopyranosyl-D-galactose and was combined with a similar fraction from the second stage of the degradation for further examination. Oligosaccharide *e* was chromatographically indistinguishable from 6-*O*-β-D-galactopyranosyl-D-galactose. The methanolysis products from the methylated disaccharide were examined by gas chromatography and the major products were shown to have the retention times of methyl glycosides of 2,3,4,6-tetra- and 2,3,4-tri-*O*-methyl-D-galactose.

Hydrolysis of the gum acid with 0·5N-sulphuric acid for 2 hr. at 100° resulted in the liberation of galactose-containing oligosaccharides. Oligosaccharide *e* was characterised as 6-*O*-β-D-galactopyranosyl-D-galactose by the formation of its crystalline phenylosazone and by the formation of 2,3,4,6-tetra- and 2,3,4-tri-*O*-methyl-D-galactose on hydrolysis of the methylated disaccharide. Oligosaccharide *g* was shown to be the polymer-homologous galactotriose, *O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-D-galactose, by the isolation of 2,3,4,6-tetra- and 2,3,4-tri-*O*-methyl-D-galactose in the proportion of 1 : 2 on hydrolysis of the methylated derivative. Paper chromatography of the sugars and their partial hydrolysis products indicated that oligosaccharides *h* and *i* were the polymer-homologous galactotetraose and galactopentaose, respectively. Oligosaccharide *f*, although apparently chromatographically homogeneous, was probably a mixture of sugars from which crystalline 3-*O*-β-D-galactopyranosyl-L-arabinose separated. Oligosaccharide *d* was tentatively identified as 4-*O*-β-D-galactopyranosyl-D-galactose on the basis of the following evidence. The sugar was chromatographically and ionophoretically indistinguishable from an authentic sample and distinct from the α-linked anomer and from 3-*O*-β-D-galactopyranosyl-D-galactose. Lead tetra-acetate oxidation of the sugar followed by hydrolysis gave a tetrose (presumably threose) which would arise only from a 4-*O*-substituted galactose residue. Gas chromatography of the methanolysis products from the methylated disaccharide indicated the presence therein as major components of methyl glycosides of 2,3,4,6-tetra- and 2,3,6-tri-*O*-methylgalactose.

In the third stage of the graded hydrolysis degraded gum B was heated with N-sulphuric acid on a boiling-water bath for 3 hr. The acidic oligosaccharides were separated from neutral sugars by adsorption on anion-exchange resin, and after desorption were fractionated by chromatography on filter sheets to give three discrete oligosaccharide components A, B, and C. Acidic oligosaccharide A was characterised as 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose by conversion into the crystalline methyl glycoside pentamethyl ether, and by reduction of the methylated derivative followed by hydrolysis to give 2,3,4-tri-*O*-methyl-D-galactose and 3,4-di-*O*-methyl-L-rhamnose. Acidic oligosaccharide B was characterised as 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose by conversion into the crystalline methyl glycoside methyl ester hexamethyl ether. Acidic oligosaccharide C was chromatographically and ionophoretically homogeneous, and chromatography of the hydrolysate showed the aldobiouronic acid, galactose, and a small amount of rhamnose. However, hydrolysis of the product from reduction with potassium borohydride showed rhamnitol and galactitol in addition to galactose, rhamnose, and the aldobiouronic acid, indicating the presence of two isomeric acidic trisaccharides, one with

rhamnose and one with galactose as the reducing unit. When the acidic fraction was treated with cold dilute alkali one component was degraded to give 2-*O*-galacturonosylrhamnose, and the other, even on extended treatment, was apparently unchanged. Assuming that both acids give rise to 2-*O*-galacturonosylrhamnose as a partial hydrolysis product, and that 2-*O*-substituted rhamnose residues are stable to the action of cold dilute alkali,[8] it is probable that the alkali-stable component contains a non-reducing galactose end-group attached to the 2-*O*-galacturonosylrhamnose moiety, and that the alkali-labile component contains the 2-*O*-galacturonosylrhamnose unit attached to a galactose reducing group by other than a 1,2-linkage. The mixture of acidic trisaccharides was methylated and the product was reduced with lithium aluminium hydride and hydrolysed to give a mixture of sugars which was shown by paper chromatography to contain tetra-, one or more tri-, and one or more di-*O*-methylgalactoses, and 3,4-di-*O*-methylrhamnose. The derived methyl glycosides were examined by gas chromatography, and components were recognised which had the retention times of methyl glycosides of 2,3,4,6-tetra-, 2,3,4- and 2,3,6-tri-*O*-methyl-D-galactose, and 3,4-di-*O*-methyl-L-rhamnose. In addition, methyl glycosides of di-*O*-methylgalactose were indicated, but in the presence of the other components it was not possible to distinguish between a single di-*O*-methylgalactose (probably the 2,3-isomer) and a mixture of this and a second component (possibly the 2,4-isomer). These results do not permit the formulation of unique structures for the components of the mixture of acidic trisaccharides, but on the assumption that 2,3,4-tri- and di-*O*-methylgalactose have arisen from the reduction of the galacturonic acid residues, structures (I) and (II) may be proposed for the two trisaccharides. The site of substitution of the galacturonic acid residue in the second component (II) is uncertain, and trisaccharides containing more than one type of linkage may be present. Since mono-*O*-methylrhamnose could not be detected as a cleavage product of the methylated trisaccharides it is certain that the mixture does not contain branched trisaccharides (*e.g.*, III).



Combretum leonense gum resembles many other plant gums [5] in containing a variety of types of glycosidic linkages which are cleaved at different rates, and especially in containing interior chains of D-galactopyranose residues. The galactose residues are mutually joined mainly by 1,6-linkages, and in this respect the gum resembles more closely the other gums from the *Combretaceae*, namely, gum ghatti [1] and *Anogeissus schimperi* gum,[2] and *Virgilia oroboides* gum,[9] rather than the gums of the *Acacia* and *Prunus* genera [5] which contain highly branched chains of D-galactopyranose residues mutually attached by 1,3- and 1,6-linkages. *Combretum leonense* gum differs also from the above-mentioned gums in containing residues of D-galacturonic acid in addition to those of D-glucuronic acid. It is probable from the present results that the residues of D-galacturonic acid in *Combretum leonense* gum are isolated units joined to other sugar residues. In contrast, the majority of other galacturonic acid-containing gums which have received detailed attention, *e.g.*, tragacanthic acid [10] and the *Khaya* gums,[11] contain contiguous residues of this sugar, either alone or interspersed with residues of L-rhamnose in the basal chains of the molecular structure.

[8] R. L. Whistler and W. M. Corbett, *J. Amer. Chem. Soc.*, 1955, **77**, 3822, 6328.
[9] A. M. Stephen, *J.*, 1957, 1919; F. Smith and A. M. Stephen, *J.*, 1961, 4892; A. M. Stephen, *J.*, 1962, 2030; 1963, 1974.
[10] S. P. James and F. Smith, *J.*, 1945, 739, 749; G. O. Aspinall and J. Baillie, *J.*, 1963, 1702, 1714.
[11] G. O. Aspinall, E. L. Hirst, and N. K. Matheson, *J.*, 1956, 989; G. O. Aspinall, M. J. Johnston, and A. M. Stephen, *J.*, 1960, 4918.

Combretum leonense gum may be compared most closely with gum ghatti,[1] *Anogeissus schimperi* gum,[2] and *Virgilia oroboides* gum [9] by considering, in turn, the acid-labile arabinose residues and units attached thereto, the chains of galactose residues, and the acidic oligosaccharide fragments which have been isolated. Table 1 indicates the oligosaccharides which have been characterised as partial hydrolysis products of these gums. In all the gums the majority of L-arabinose residues are removed by mild acid hydrolysis; these are present in the outer chains of the polysaccharides and it is shown in the following Paper [4] that in *Combretum leonense* gum the majority of these are present as single unit end-groups in the furanose form. Mild acid hydrolysis of the gum, as of *A. schimperi* and *V. oroboides* gums, also liberates arabinose-containing oligosaccharides, and the nature of these fragments varies considerably between the different gums. Gum ghatti [12] probably also contains contiguous L-arabinose residues in the outer chains, but no arabinobioses have yet been characterised as partial hydrolysis products of the gum. Gum ghatti and *A. schimperi* gum also contain L-arabinose residues in the interior chains since partial hydrolysis of each gum gives rise to the homologous series of oligosaccharides, *O*-β-D-galactopyranosyl-[(1 → 6)-*O*-β-D-galactopyranosyl]$_n$-(1 → 3)-L-arabinose. In the case of gum ghatti [12] it is probable that some of the arabinose units in these oligosaccharides arise from interior 3-*O*-substituted L-arabinopyranose residues. *Combretum leonense* gum furnishes 3-*O*-β-D-galactopyranosyl-L-arabinose as a minor product of partial hydrolysis, but none of the higher members of the oligosaccharide series could be detected. Since the gum contains 3-*O*-substituted L-arabinose residues in the furanose form only,[4] it is probable that the disaccharide arises from a peripheral unit rather than from interior chains.

TABLE 1

Oligosaccharides

Gum	Periphery	Galactan framework	Acidic fragments
Anogeissus latifolia		Gal*p* 1β → 6 Gal Gal*p* 1β → 6 Gal*p* 1β → 6 Gal Gal*p* 1β → 3 Gal Gal*p* 1β → 3 Ara Gal*p* 1β → 6 Gal*p* 1β → 3 Ara	Gp A 1β → 6 Gal Gp A 1β → 2 Man Gp A 1β → 2 Man*p* 1β → 2 Man
Anogeissus schimperi	Ara*f* 1β → 3 Ara Arap 1β → 3 Ara	Gal*p* 1β → 6 Gal Gal*p* 1β → 6 Gal*p* 1β → 6 Gal Gal*p* 1β → 3 Gal Gal*p* 1β → 3 Ara Gal*p* 1β → 6 Gal*p* 1β → 3 Ara	Gp A 1β → 6 Gal Gp A 1β → 2 Man
Combretum leonense	Ara*p* 1β → 3 Ara Gal*p* 1β → 3 Ara	Gal*p* 1β → 6 Gal Gal*p* 1β → 6 Gal*p* 1β → 6 Gal Gal*p* 1β → 4 Gal	Gp A 1β → 6 Gal Gal*p* A 1α → 2 Rha
Virgilia oroboides	Ara*f* 1α → 3 Ara Ara*f* 1α → 5 Ara Ara*p* 1α → 5 Ara Xyl*p* 1β → 5 Ara	Gal*p* 1β → 6 Gal Gal*p* 1β → 6 Gal*p* 1β → 6 Gal Gal*p* 1β → 3 Gal	Gp A 1β → 6 Gal Gp A 1β → 2 Man

All four gums under discussion contain chains of 1,6-linked D-galactopyranose residues as an important part of the molecular structure, and in the case of the *Anogeissus* gums some, at least, of these chains are attached to L-arabinose residues. In the *Anogeissus* and *Virgilia* gums some D-galactose residues are mutually joined by 1,3-linkages, but in *Combretum leonense* gum this type of linkage has not been encountered, but is probably replaced by a small proportion of 1,4-linkages.

Interesting variations in the structures of the four gums are indicated in the acidic oligosaccharides which are formed on partial hydrolysis. All the gums afford 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, and the *Anogeissus* and *Virgilia oroboides* gums furnish 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose although the relative amounts of the two aldobiouronic acids vary between the gums. In the case of *Combretum leonense*

[12] G. O. Aspinall, V. P. Bhavanandan, and T. B. Christensen, preceding Paper.

gum the mannose-containing aldobiouronic acid is replaced by 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose. Higher acidic oligosaccharides have been isolated from some of the gums, but, except in the case of gum ghatti,[12] there is no information concerning the structural relationship between the various acidic fragments or of the relationship of the acidic with the neutral portions of the molecular structure.

Experimental

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) ethyl acetate–acetic acid–formic acid–water (18 : 8 : 3 : 9); (D) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (E) butan-1-ol–acetic acid–water (4 : 1 : 5, upper layer); (F) benzene–ethanol–water (11 : 3 : 1, upper layer); (G) butan-2-one, half saturated with water; (H) butan-1-ol–ethanol–water (1 : 1 : 1, upper layer); (I) butan-2-one–acetic acid–water (9 : 1 : 1, saturated with boric acid). R_G Values of methylated sugars refer to rates of movement relative to 2,3,4,6-tetra-*O*-methyl-D-glucose in solvent D. Demethylations of methylated sugars were performed with hydriodic acid.[13] Chromatography of the periodate oxidation products of methylated sugars was carried out by Lemieux and Bauer's method.[14] Unless otherwise stated, optical rotations were observed for water solutions at *ca.* 18°.

Gas chromatography of methylated and partially methylated methyl glycosides was carried out using a Pye Argon Chromatograph according to the procedure of Bishop and Cooper [6] (see also ref. 7). Separations were made on the following columns (120 × 0·5 cm. i.d.) at gas flow rates of 80—100 ml./min.: (*a*) 15% by weight of butane-1,4-diol succinate polyester [6] on acid-washed Celite (80—100 mesh) at 175°; (*b*) 10% by weight of polyphenyl ether [*m*-bis-(*m*-phenoxyphenoxy)benzene] on acid-washed Celite at 200°. Retention times (*T*) are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside as an internal standard.

Crushed gum nodules (30 g.) were dissolved in water (600 ml.), mechanical impurities were removed, concentrated hydrochloric acid (18 ml.) was added slowly with stirring, and the gum acid was precipitated by the addition of ethanol (2·5 l.). The dried gum acid (23 g.) had $[\alpha]_D$ −15·9° (*c* 4·0 in 0·1N-NaOH) and uronic anhydride (by decarboxylation), 15·2%. A second batch of gum nodules (40 g.) afforded gum acid (32 g.), $[\alpha]_D$ −9·8° (*c* 1·0 in 0·1N-NaOH) and uronic anhydride, 13·9%.

Partial Hydrolysis of the Gum (1).—The gum acid (20 g.) was heated in water (200 ml.) on a boiling-water bath for 36 hr. (constant rotation). The cooled solution was neutralised with barium hydroxide and barium carbonate, filtered, and poured into ethanol (4 vol.). The precipitated polysaccharide was removed at the centrifuge, washed with ethanol, dissolved in water (200 ml.), and re-precipitated to give degraded gum A (as barium salt) (10·5 g.), which had $[\alpha]_D$ +51·3° (*c* 1·13) and uronic anhydride, 25·7%. The combined supernatant liquid and ethanol washings were concentrated (200 ml.), passed through successive columns of Amberlite resins IR-120(H) and IR-45(OH) to remove barium ions and traces of acidic sugars, and concentrated to a syrup (9·2 g.). The syrup was placed on charcoal–Celite (30 × 5·5 cm.) and the column was eluted with water and then with water containing increasing concentrations of ethanol. Elution with water gave fraction 1 (7·96 g.) which contained monosaccharides and small amounts of disacchrides. Further elution with water gave fraction 2 (0·585 g.) which contained monosaccharides and larger amounts of various oligosaccharides. Elution with water containing 5% of ethanol gave fraction 3 (0·143 g.) which contained chromatographically pure galactotriose ($R_{galactose}$ 0·68 in solvent H). Elution with water containing 20% of ethanol gave fraction 4 (0·65 g.) which contained higher oligosaccharides which were not examined further. Fraction 1 was refractionated on charcoal–Celite to give fraction 1a (6·64 g.) which contained monosaccharides, fraction 1b (71 mg.) which contained oligosaccharide *b* with traces of other sugars, and fraction 1c (0·34 g.) which contained a mixture of oligosaccharides. Fractions 1c and 2 were combined (0·95 g.) and separated on cellulose (44 × 2 cm.) using butan-1-ol half saturated with water as eluant, to give fractions *i*—*vi*. Fraction *i* (310 mg.) contained arabinose and rhamnose, fraction *ii* (76 mg.) contained galactose, arabinose, and oligosaccharides *a* and *b*, fraction *iii* (25 mg.) contained oligosaccharide *b* and traces of other

[13] L. Hough, J. K. N. Jones, and W. H. Wadman, *J.*, 1950, 1702.
[14] R. U. Lemieux and H. F. Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

sugars, fraction *iv* (67 mg.) contained oligosaccharides *b* and *c*, fraction *v* (16 mg.) contained oligosaccharide *d* and traces of other sugars, and fraction *vi* (288 mg.) contained oligosaccharide *e*.

A sample of oligosaccharide *a*, containing a trace of galactose, was isolated from fraction *ii* by separation on filter sheets using solvents A and B; it had $R_{galactose}$ 1·2 and $[\alpha]_D$ +28° (*c* 0·36), and gave arabinose on hydrolysis. Chromatographically pure oligosaccharide *b* (50 mg.), $R_{galactose}$ 0·82 and 0·68 in solvents A and B and $[\alpha]_D$ +164° (*c* 1·0), was isolated from fractions 1b and *iii* by chromatography on filter sheets in solvent B. The sugar was characterised as 3-*O*-β-L-arabinopyranosyl-L-arabinose by conversion into the phenylosazone, which was identified by m. p. and mixed m. p. 225—228°, $[\alpha]_D$ +42° (*c* 0·07 in Me_2CO), and *X*-ray powder photograph, and by oxidation with periodate to give formaldehyde, but no mesoxaldehyde 1,2-bisphenylhydrazone.[15] The sugar (20 mg.) was methylated with methyl sulphate and sodium hydroxide, and with methyl iodide and silver oxide, to give the methylated disaccharide (21 mg.). A portion (2 mg.) of the methylated derivative was heated with 2% methanolic hydrogen chloride in a sealed tube at 100° for 15 hr., and the product was examined by gas chromatography on columns *a* and *b*. Components were observed which had the retention times of methyl glycosides of 2,3,4-tri-*O*-methyl-L-arabinose (*T* 2·18 and 2·29, and 1·10 and 1·14), and, in small amount, of 2,5-di-*O*-methyl-L-arabinose (*T* 1·88 and 3·39, and 0·71 and 1·02). The remainder of the methylated disaccharide (19 mg.) was hydrolysed with N-hydrochloric acid on a boiling-water bath for 4 hr., and the products were separated on a filter sheet in solvent D to give fractions *a* and *b*. Fraction *a* (8 mg.) was chromatographically identical with 2,3,4-tri-*O*-methyl-L-arabinose, but attempts to form a crystalline derivative failed, and fraction *b* (7 mg.) was characterised as 2,4-di-*O*-methyl-L-arabinose by conversion into the aniline derivative, m. p. and mixed m. p. 120—122°. Oligosaccharide *c* (9 mg.), $R_{galactose}$ 0·59 in solvent A and $[\alpha]_D$ +53 (*c* 0·46), was isolated in chromatographically pure form from fraction *iv* and had the same mobility as 3-*O*-β-D-galactopyranosyl-L-arabinose. Hydrolysis of the sugar gave galactose and arabinose, and hydrolysis of the derived glycitol (from borohydride reduction) gave arabinose and galactitol with traces of galactose and arabitol. Oligosaccharide *d* (6 mg.), which was isolated from fraction *v*, had the same chromatographic mobility as 4-*O*-β-D-galactopyranosyl-D-galactose in solvents A and B and was combined with material from fraction C (stage 2 of the partial hydrolysis) for further examination. Oligosaccharide *e* (from fraction *vi*) was chromatographically indistinguishable from 6-*O*-β-D-galactopyranosyl-D-galactose, which was characterised as a product of stage 2 of the partial hydrolysis. A sample of this fraction was methylated with methyl sulphate and sodium hydroxide, and with methyl iodide and silver oxide, and the methanolysis products from the methylated derivative were examined by gas chromatography on column *a*. Although a number of minor components were present, the major components had the retention times of methyl glycosides of 2,3,4,6-tetra- (*T* 1·80) and 2,3,4-tri-*O*-methyl-D-galactose (*T* 7·5).

Partial Hydrolysis of the Gum (2).—The gum acid (30 g.) was heated with 0·5N-sulphuric acid (450 ml.) on a boiling-water bath for 2 hr. The cooled solution was neutralised with barium hydroxide and barium carbonate, filtered, concentrated, and poured into ethanol (4 vol.). Degraded gum B (10 g.) was removed at the centrifuge, and the supernatant liquid was concentrated to a syrup (16·5 g.). The syrup (16·5 g.) was dissolved in water and adsorbed on charcoal–Celite (1 : 1; 50 × 7 cm.). Elution with water gave mainly monosaccharides (11·5 g.). Elution with water containing 2·5% of ethanol gave fraction A (0·5 g.) containing oligosaccharides *e* and *f* and fraction B (130 mg.) containing oligosaccharides *e*, *f*, and *g*. Elution with water containing 5% of ethanol gave fraction C (80 mg.) containing oligosaccharides *d*, *e*, and *g*, and fraction D (332 mg.) containing oligosaccharides *g* (major component) and *h*. Elution with water containing 10% of ethanol gave fraction E (512 mg.) containing oligosaccharides *e*, *g*, *h*, and *i*. The various oligosaccharides were isolated after chromatographic separations on filter sheets.

Oligosaccharide d (from fractions *v* and C), $[\alpha]_D$ +28° (*c* 0·82) and $R_{galactose}$ 0·49 and 0·40 in solvents A and B, was chromatographically and ionophoretically indistinguishable from 4-*O*-β-D-galactopyranosyl-D-galactose but different from 4-*O*-α-D-galactopyranosyl-D-galactose and 3-*O*-β-D-galactopyranosyl-D-galactose. Oxidation of the sugar with lead tetra-acetate followed by hydrolysis[16] gave galactose and a tetrose (presumably threose) whereas similar

[15] L. Hough, D. B. Powell, and B. M. Woods, *J.*, 1956, 4799.
[16] A. S. Perlin, *Analyt. Chem.*, 1955, **27**, 396.

treatment of 3-*O*-β-D-galactopyranosyl-D-galactose gave galactose and lyxose. The sugar (4 mg.) was methylated successively with methyl sulphate and sodium hydroxide, and methyl iodide and silver oxide, and the methanolysis products from the methylated derivative were examined by gas chromatography. A complex mixture of products was observed, but components having the retention times of methyl glycosides of 2,3,4,6-tetra- (*T* 1·80, and 1·53 and 1·61 on columns *a* and *b*) and 2,3,6-tri-*O*-methyl-D-galactose (*T* 3·24 and 4·73, and 1·61, 2·07, 2·24, and 2·53 on columns *a* and *b*) were recognised as major components.

Oligosaccharide e (from fraction A) had $[\alpha]_D$ +30° (*c* 0·15) and $R_{galactose}$ 0·23 in solvent A, and was chromatographically indistinguishable from 6-*O*-β-D-galactopyranosyl-D-galactose. Hydrolysis of the sugar gave galactose only. The sugar furnished a phenylosazone identical with that from 6-*O*-β-D-galactose (m. p. and mixed m. p. 179—181°, and *X*-ray powder photograph). Methylation of the disaccharide (100 mg.) with methyl sulphate and sodium hydroxide, and methyl iodide and silver oxide, gave the methylated disaccharide (60 mg.). Hydrolysis of the methylated derivative (42 mg.) gave 2,3,4,6-tetra-*O*-methyl-D-galactose (10 mg.) (aniline derivative, m. p. and mixed m. p. 189—190°), 2,3,4-tri-*O*-methyl-D-galactose (12 mg.) (aniline derivative, m. p. 165—166° and mixed m. p. 163—165°), and a small amount (3 mg.) of di-*O*-methylgalactose from incomplete etherification.

Oligosaccharide f (from fraction A), $[\alpha]_D$ +22° (*c* 1·25) and $R_{galactose}$ 0·59 and 0·53 in solvents A and B, gave galactose and arabinose on hydrolysis. Part of the syrup crystallised, and after recrystallisation from ethanol–water was identical with 3-*O*-β-D-galactopyranosyl-L-arabinose (m. p. and mixed m. p. 175—177°, and *X*-ray powder photograph).

Oligosaccharide g (from fraction D), $[\alpha]_D$ +12·6° (*c* 1·5) and $R_{galactose}$ 0·68 in solvent H, was chromatographically indistinguishable from *O*-β-D-galactopyranoyl-(1 → 6)-*O*-β-D-galactopyranosyl-(1 → 6)-D-galactose in solvents A, B, and H. Partial acid hydrolysis gave galactose and 6-*O*-β-galactopyranosylgalactose. Methylation of the sugar (150 mg.) with methyl sulphate and sodium hydroxide, and methyl iodide and silver oxide, furnished the methylated derivative (102 mg.), hydrolysis of which gave 2,3,4,6-tetra-*O*-methyl-D-galactose (35 mg.) (aniline derivative, m. p. and mixed m. p. 190—191°), and 2,3,4-tri-*O*-methyl-D-galactose (59 mg.) [aniline derivative, m. p. 160—162° and mixed m. p. (with sample, m. p. 169°) 165—166°].

Oligosaccharide h (from fraction E), $[\alpha]_D$ +11·1° (*c* 2·7) and $R_{galactose}$ 0·26 in solvent H, was chromatographically indistinguishable from the 1,6-linked galactotetraose from gum ghatti [1] and *Anogeissus schimperi* gum,[2] and gave galactose and 6-*O*-β-galactopyranosylgalactose on partial acid hydrolysis.

Oligosaccharide i (from fraction E), $[\alpha]_D$ +7·1° (*c* 1·1) and $R_{galactose}$ 0·15 in solvent H, was chromatographically indistinguishable from the 1,6-linked galactopentaose from gum ghatti [1] and *Anogeissus schimperi* gum,[2] and gave galactose and 6-*O*-β-galactopyranosylgalactose on partial acid hydrolysis.

Partial Hydrolysis of the Gum (3).—Degraded gum B (9·5 g.) was heated with N-sulphuric acid on a boiling-water bath for 3 hr., and the cooled solution was neutralised with barium carbonate, filtered, passed through Amberlite resin IR-120(H) to remove barium ions, and concentrated to a syrup (5·65 g.). The syrup was poured on to a column of Amberlite resin IR-45 (formate form) to adsorb acids, and elution with water removed neutral sugars (3·03 g.). Elution of the column with 0·5% formic acid afforded a mixture of acidic sugars (1·99 g.) which were separated on filter sheets using solvent C to give three main fractions.

Fraction 1 (0·635 g.) had $[\alpha]_D$ +66·3° (*c* 3·4) and $R_{galacturonic\ acid}$ 0·79 and 0·82 in solvents B and C. Reduction of the derived 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 (459 mg.),[17] a sample of which was recrystallised from light petroleum (b. p. 100—120°) and was identified by m. p. 67—70° and mixed m. p. 70—71°, $[\alpha]_D$ +94° (*c* 1·33 in $CHCl_3$), and *X*-ray powder photograph. The remainder of the methylated aldobiouronic acid was reduced with diborane in 1,2-dimethoxyethane,[17] a portion (150 mg.) of the product was hydrolysed with N-hydrochloric acid on a boiling-water bath for 4 hr., and the solution was neutralised with Amberlite resin IR-4B(OH), and concentrated to a syrup which was separated on cellulose (50 × 2 cm.), light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3), saturated with water, being used as eluant, to give two main fractions with a minor

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

fraction which contained a mixture of sugars (8 mg.). The sugars were characterised as (*a*) 3,4-di-*O*-methyl-L-rhamnose (62 mg.), R_G 0·90, m. p. 86° and mixed m. p. (with sample, m. p. 89—90°) 88—90°, and (*b*) 2,3,4-tri-*O*-methyl-D-galactose (85 mg.), R_G 0·72 [aniline derivative, m. p. 159—160° and mixed m. p. (with sample m. p. 165—166°) 165—166°].

Fraction 2 (0·305 g.) had $[\alpha]_D$ +21·4° (*c* 2·1) and $R_{\text{galacturonic acid}}$ 0·41 and 0·54 in solvents B and C. Reduction of the derived methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave glucose, galactose, and a trace of rhamnose (probably due to slight contamination with fraction 3). The aldobiouronic acid was characterised as 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose by conversion into the methyl ester methyl glycoside hexamethyl ether,[18] which was identified by m. p. and mixed m. p. 81—83°, $[\alpha]_D$ −16·2° (*c* 0·8 in $CHCl_3$), and *X*-ray powder photograph.

Fraction 3 (116 mg.) had $[\alpha]_D$ +63° (*c* 0·64) and only one component was indicated by chromatography in solvents B and C ($R_{\text{galacturonic acid}}$ 0·16 and 0·27) and ionophoresis. Hydrolysis gave 2-*O*-galacturonosylrhamnose, galactose, and some rhamnose. Reduction of the derived methyl ester methyl glycosides followed by hydrolysis gave galactose and rhamnose. Reduction of the potassium salt of the sugar acid with potassium borohydride followed by hydrolysis gave galactitol and rhamnitol, in addition to galactose, rhamnose, and 2-O-galacturonosylrhamnose. A sample of the sugar acids was treated with oxygen-free N-sodium hydroxide for 2 weeks, the solution was neutralised with Amberlite resin IR-120(H) and concentrated, and examination of the resulting syrup showed components having the chromatographic mobilities of unchanged starting material and 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose. No further change was observed when the sugar acid was treated with alkali for another 2 weeks. Under the same conditions 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose was apparently undegraded by alkali. The sugar acids (25 mg.) were methylated with six additions of methyl sulphate and 30% sodium hydroxide, and extraction of the acidified solution with chloroform gave a syrup which was reduced with lithium aluminium hydride in tetrahydrofuran, and hydrolysed with N-sulphuric acid on a boiling-water bath for 6 hr. Paper-chromatographic examination in solvents D, F, and G of the resulting mixture of sugars showed tetra-, one or more tri-, and one or more di-*O*-methylgalactoses, and 3,4-di-*O*-methylrhamnose. The sugars were converted into their methyl glycosides which were examined by gas chromatography on columns *a* and *b*. As indicated in Table 2, the mixture contained components having the retention times of methyl glycosides of 3,4-di-*O*-methyl-L-rhamnose, 2,3,4,6-tetra-, and 2,3,4- and 2,3,6-tri-*O*-methyl-D-galactose on both columns, and of 2,3- and/or 2,4-*O*-methyl-D-galactose on column *b*.

TABLE 2

Relative retention times of methyl glycosides

Methyl ether	Column *a*		Column *b*	
	Authentic sample	Mixture	Authentic sample	Mixture
3,4-Di-*O*-methyl-L-rhamnose	0·73 1·01	0·73 1·02	0·61	0·61
2,3,4,6-Tetra-*O*-methyl-D-galactose	1·80	1·80	1·52 1·61	1·52 (1·62)
2,3,4-Tri-*O*-methyl-D-galactose	7·5	7·5	2·64 2·90	2·66 2·94
2,3,6-Tri-*O*-methyl-D-galactose	3·22 3·93 4·30 4·70	3·28 3·95 4·22 4·76	1·61 2·07 2·23 2·49	(1·62) 2·07 2·28 (2·50)
2,3-Di-*O*-methyl-D-galactose			2·46 3·19 3·65 4·20	(2·50) 3·21 (3·74) (4·41)
2,4-Di-*O*-methyl-D-galactose			3·72 4·40	(3·74) (4·41)

Figures in parentheses indicate relative retention times of components which were incompletely resolved.

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[18] S. W. Challinor, W. N. Haworth, and E. L. Hirst, *J.*, 1931, 258.