488. Combretum leonense *Gum. Part II.*[1] *Hydrolysis Products from the Methylated Gum and the Methylated Arabinose-free Degraded Gum*

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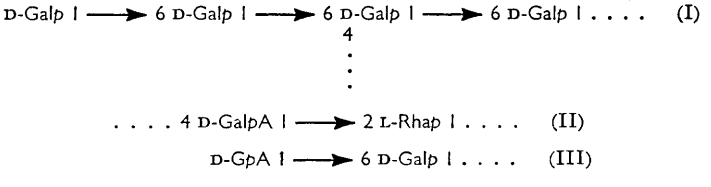
The complex mixtures of sugars, which are formed on hydrolysis of methylated *Combretum leonense* gum and of the methylated arabinose-free degraded gum, have been characterised. Partial structures for the gum are advanced in the light of these and previous experiments. The nature of the heterogeneity of the gum has been assessed by examination of the polysaccharide fractions isolated from chromatography on diethylaminoethylcellulose.

It was shown in Part I[1] that *Combretum leonense* gum can be degraded in a stepwise manner with the release of monosaccharides and various neutral and acidic oligosaccharides. Autohydrolysis of the gum resulted in the preferential release of arabinose residues, accompanied by a small proportion of galactose residues, with the formation of a degraded gum which was almost devoid of arabinose residues.[1] This degraded gum has now been converted into its methylated derivative. Hydrolysis of the methylated degraded gum gave a mixture of neutral and acidic sugars. The neutral methylated sugars were separated by column chromatography and the following sugars were characterised by derivative formation or by one or more of the following criteria, optical rotation, chromatography and paper ionophoresis of the sugars, chromatography of the products of demethylation and of periodate oxidation, and gas chromatography of the derived methyl glycosides: 2,3,4,6-tetra-, 2,3,4-tri-, and 2,3-di-*O*-methyl-D-galactose, and, in smaller amount, 3,4-di- and 3-*O*-methyl-L-rhamnose. Traces of the following sugars were also detected: 2,3,4-tri-*O*-methylarabinose, 2,3,6-tri-, 2,4- and 3,4-di-, and 2,*O*-methylgalactose, galactose, rhamnose, and 2,3,4-tri-*O*-methylglucuronic acid. The acidic sugars were converted into the methyl ester methyl glycosides, reduced with lithium aluminium hydride, and hydrolysed to give 2,3,4-tri-*O*-methyl-D-glucose, 3,4-di- and 3-*O*-methyl-L-rhamnose, and 2,3,4-tri-, 2,3-di-, and, in trace amount, 2-*O*-methyl-D-galactose.

The significance of these results may be considered in terms of the oligosaccharide fragments formed on partial acid hydrolysis of the gum.[1] Most of the neutral sugars from the methylated degraded gum have their origin in the 1,6-linked chains of β-D-galactopyranose residues, but the isolation of 2,3-di-*O*-methyl-D-galactose shows that some of the residues carry side-chains at C-4 as in (I). It is probable that the acidic fraction obtained on hydrolysis of the methylated degraded gum consisted largely of fully or partially methylated derivatives of the aldobiouronic acids, 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose and 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose. Since 2,3-di-*O*-methyl-D-galactose and the mixture of 3,4-di- and 3-*O*-methyl-L-rhamnose are



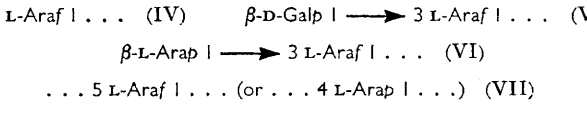
formed in approximately equimolecular proportions as the major components of the mixture of sugars derived from the acidic fraction, it is probable that the former sugar arises from 4-*O*-substituted D-galacturonic acid residues in disaccharide units (II) in which the majority of L-rhamnose residues are present as branching points. The D-glucuronic acid

[1] Part I, preceding Paper.

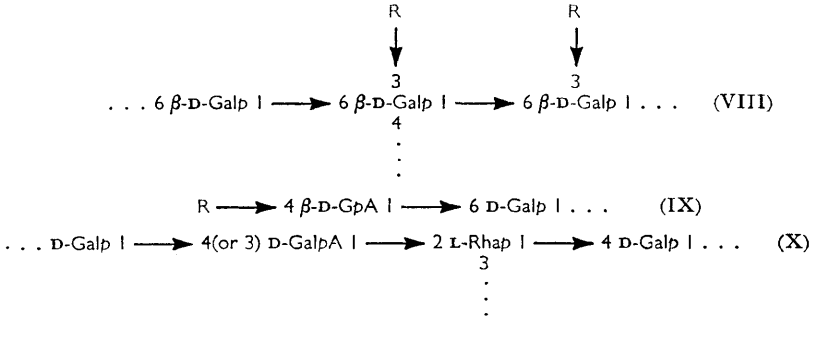
residues in the degraded gum are clearly present as end-groups which give rise to 2,3,4-tri-*O*-methyl-D-glucose. Since methyl ethers of D-galactose may be formed in the above sequence of reactions both from D-galactose residues originally present in the polysaccharide and from the reduction of D-galacturonic acid residues, it is not possible to ascribe unique structural significance to 2,3,4-tri-*O*-methyl-D-galactose. If, however, the former alternative is assumed, it follows that the D-glucuronic acid residues terminate side-chains units as in (III).

Methylated *Combretum leonense* gum was hydrolysed in a similar manner and the products were separated into neutral and acidic fractions. The following neutral sugars were characterised by derivative formation: 2,3,5-tri-, 2,3- and 2,5-di-*O*-methyl-L-arabinose, 2,3,4- and 2,3,6-tri-, 2,3-, 2,4-, and 2,6-di-, and 2-*O*-methyl-D-galactose, 3-*O*-methyl-L-rhamnose, and L-rhamnose. In addition, the following sugars, all present in very small amount, were recognised on the basis of chromatography of the sugars and their derivatives, and of gas chromatography of the derived methyl glycosides: 2,3,4-tri-*O*-methylarabinose, 2,3,4,6-tetra- and 3-*O*-methylgalactose, and 3,4-di-*O*-methylrhamnose. The acidic sugars were converted into the methyl ester methyl glycosides, reduced with lithium aluminium hydride, and hydrolysed to give 2,3,4-tri- and 2,3-di-*O*-methyl-D-galactose, 2,3-di-*O*-methyl-D-glucose, and 3-*O*-methyl-L-rhamnose, with traces of 2,4-di-*O*-methylgalactose and rhamnose.

The structural significance of these cleavage products may be assessed by considering the nature of the acid-labile L-arabinose residues which are removed by autohydrolysis in the preparation of the degraded gum and the differences between the substitution patterns of the more acid-stable portions (I, II, and III) of the molecular structure in the degraded gum and the corresponding units in the original gum. The majority of the peripheral arabinose units in the gum occur as L-arabinofuranose end-groups (IV). Since 3-*O*-β-D-galactopyranosyl-L-arabinose and 3-*O*-β-L-arabinopyranosyl-L-arabinose were isolated as partial hydrolysis products of the gum,[1] and since 2,5-di-*O*-methyl-L-arabinose (but not the 2,4-dimethyl ether) was formed on hydrolysis of the methylated gum, it follows that the disaccharides originate from units (V) and (VI) which are probably present in the outer chains. The isolation of 2,3-di-*O*-methyl-L-arabinose as a minor product from the hydrolysis of the methylated gum points to the presence of other non-terminal L-arabinose residues (VII) in the gum.



The isolation from the methylated gum of 2,3,4-tri-, 2,4-di-, and 2-*O*-methyl-D-galactose in approximately equimolecular proportions indicates that the majority of the acid-labile groups (R = IV—VII) are attached to C-3 of 1,6-linked β-D-galactopyranose residues as in (VIII). The presence of 2,3-di-*O*-methyl-D-glucuronic acid residues in the methylated gum



shows that the D-glucuronic acid residues in the gum are 4-*O*-substituted and are probably present in the partial structure (IX). The evidence for the attachment of acid-labile substituents to the other aldobiouronic acid units (II) is less certain, although some such substituents may be linked to L-rhamnose residues. The isolation, however, of two isomeric trisaccharides from the partial hydrolysis of the gum [1] suggests that these aldobiouronic acid units are flanked on either side by D-galactose residues, and partial structure (X) may be proposed for this part of the molecular structure. The gum also contains a small proportion of continguous D-galactopyranose residues joined by 1 ⟶ 4 linkages, as shown by the isolation of the corresponding galactobiose as a minor product of partial hydrolysis, but the location of these units in the molecular structure is not yet known.

The major structural features of *Combretum leonense* gum may be summarised in the partial structures (VIII), (IX), and (X), to which the various acid-labile groups (R) are attached at the sites indicated. Since Anderson, Hirst, and King [2] had shown previously that the polysaccharide samples isolated from individual nodules of the gum showed certain variations in composition, notably in uronic anhydride content (*ca.* 15—20%), the possibility that the gum might contain a mixture of polysaccharides of different structural types was explored. Although gum nodules of adequate size were no longer available for the further study of individual nodules, a sample of the gross polysaccharide preparation, on which the detailed structural investigations had been carried out, was fractionated by chromatography on diethylaminoethylcellulose.[3] Two polysaccharide fractions were obtained whose uronic anhydride contents corresponded to those of the most widely differing polysaccharide samples which had been isolated from individual nodules.[2] In addition, a small amount of neutral polysaccharide was isolated; it is possible that this material, which contained 1,6-linked galactose residues with attached arabinose residues, resulted from inadvertent degradation of the gum. The products of partial acid hydrolysis of the two polysaccharide fractions were examined by paper chromatography, and in each case sugars with the chromatographic mobilities of arabinose, rhamnose, galactose, 6-*O*-β-D-galactopyranosyl-D-galactose, 3-*O*-β-D-galactopyranosyl-L-arabinose, and the two aldobiouronic acids, 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose and 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, were detected. Polysaccharide samples were methylated and the methanolysis products from the derivatives were examined by gas chromatography. The cleavage products from the two methylated polysaccharide samples contained qualitatively similar mixtures of components amongst which methyl glycosides of the following sugars were recognised by their retention times: 2,3,5-tri-, and 2,3- and 2,5-di-*O*-methylarabinose, 2,3,4,6-tetra-, 2,3,4- and 2,3,6-tri-, and 2,4-di-*O*-methylgalactose, 3-*O*-methylrhamnose, and 2,3-di-*O*-methylgalacturonic acid. Ultracentrifugation of the two polysaccharide samples (by courtesy of Dr. C. T. Greenwood) showed in each case a single peak with the same sedimentation coefficient. These results, therefore, provide strong evidence against the presence in the gum of polysaccharides of structurally different types, and suggest that the gum contains a mixture of polysaccharides composed of the same structural units which are linked in a similar manner but are present in slightly differing proportions. This type of micro-heterogeneity may be contrasted with the gross heterogeneity of gum tragacanth [4] and *Khaya senegalensis* gum [5] where each gum contains a mixture of structurally unrelated polysaccharides.

Experimental

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

Preparation and Hydrolysis of Methylated Degraded Arabinose-free Gum.—Degraded arabinose-free gum A [1] (9 g.) was methylated successively with methyl sulphate and sodium hydroxide,

[2] D. M. W. Anderson, E. L. Hirst, and N. J. King, *Talanta*, 1959, **3**, 118.
[3] H. Neukom, H. Deuel, W. J. Heri, and W. Kündig *Helv. Chim. Acta*, 1960, **43**, 67.
[4] S. P. James and F. Smith, *J.*, 1945, 739, 749; G. O. Aspinall and J. Baillie, *J.*, 1963, 1702, 1714.
[5] G. O. Aspinall, M. J. Johnston, and A. M. Stephen, *J.*, 1960, 4918; G. O. Aspinall, M. J. Johnston, and R. Young, following Paper.

and methyl iodide and silver oxide, to give methylated degraded gum (2·98 g.) (Found: OMe, 42·8%, not raised on further methylation). The methylated derivative was suspended in 2N-sulphuric acid (100 ml.) at room temperature for 5 days and at 50° for 1 day. The resulting solution was diluted to 200 ml., heated on a boiling-water bath for 10 hr. (constant rotation), cooled, and neutralised with barium hydroxide and barium carbonate. The filtrate and washings were concentrated to a syrup (1·7 g.) which was fractionated on cellulose (45 × 2·5 cm.) with butan-1-ol half saturated with water, to give fractions A (820 mg.), which contained neutral methylated sugars, B (83 mg.), which contained tetra- and tri-*O*-methylgalactose and acidic sugars and was not examined further, and C (103 mg.), which contained acidic methylated sugars. Further elution of the column with water gave fraction D (637 mg.), which contained the barium salts of acidic methylated sugars. The syrup A (820 mg.) was separated on cellulose (55 × 1·5 cm.), (i) light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3, later 1 : 1), saturated with water, (ii) butan-1-ol, half saturated with water, and (iii) water being used as eluants to give eleven fractions. Fractions 6 and 11 contained mixtures of acidic methylated sugars which were chromatographically similar to those in fraction C. The combined fractions C, 6, and 11 were converted into the acidic form by removal of barium ions with Amberlite resin IR-120(H), converted into the methyl ester methyl glycosides with methanolic hydrogen chloride, reduced with lithium aluminium hydride, and hydrolysed with N-sulphuric acid to give a syrup C′ (110 mg.). Fraction D (637 mg.) was treated in a similar manner to give a syrupy mixture D′ (208 mg.) of methylated sugars. The syrups C′ and D′ were examined by paper chromatography in solvents A, D, and F, and by gas chromatography of the methyl glycosides, and were shown

TABLE 1

Analysis of hydrolysate of methylated arabinose-free degraded gum

Fraction	Wt. (mg.)	$[\alpha]_D$	Paper chromatography * R_G	Sugar	Sugars given on demethylation	Other evidence †
1	135	+84°	0·86	Me_4 galactose 3,4-Me_2 rhamnose		
2	7	+95	0·84 0·73	2,3,4-Me_3 arabinose 2,3,6-Me_3 galactose	Arabinose Galactose	F, G
3	243	+118	0·73	2,3,4-Me_3 galactose		F, G, g.l.c.
4	41	+67	0·77 0·66	2,3,4-Me_3 galactose 3-Me rhamnose		F, G
5	8	+25	0·68	3-Me rhamnose		F, G
6	12			Methylated acids		
7	46	+81	0·50	2,3-Me_2 galactose		I, P
8	7		0·48	2,3-Me_2 galactose 2,4-Me_2 galactose		I, P
9	23		0·43 0·35	2,3-Me_2 galactose (t) 3,4-Me_2 galactose (t) Rhamnose		B, I, P
10	30		0·30 0·14 Streak	2-Me galactose Galactose Methylated acid		
11	28			Barium salts		
12	38	+45	0·86	2,3,4-Me_3 glucose 3,4-Me_2 rhamnose		F, G, I
13	32	+125	0·72	2,3,4-Me_3 galactose		F
14	45	+38	0·67	3-Me rhamnose		F
15	93	+78	0·67 0·52 0·35	3-Me rhamnose (t) 2,3-Me_2 galactose Rhamnose		F, I, P
16	14		0·52 0·29	2,3-Me_2 galactose 2-Me galactose		F, G, I, P

* t = trace. † B, F, and G = paper chromatography in solvents B, F, and G. I = paper ionophoresis in borate buffer. P = paper chromatography of the periodate-oxidised sugar. g.l.c. = gas chromatography of the methyl glycosides.

to contain 2,3,4-tri-*O*-methylglucose, 2,3,4-tri- and 2,3-di-*O*-methylgalactose, 3,4-di- and 3-*O*-methylrhamnose. The relatively higher proportions of 2,3,4-tri-*O*-methylglucose and 2,3,4-tri-*O*-methylgalactose in the syrup C′ suggested that these two sugars may have arisen from the reduction and hydrolysis of a methylated aldobiouronic acid. The remainder (285 mg.) of the

syrups C′ and D′ were combined and separated on cellulose as described previously to give fractions 12—16. Table 1 summarises the results of preliminary examination of the various fractions.

Fraction 1. The syrup (130 mg.) was separated on filter sheets using solvent F, to give pure samples of 3,4-di-*O*-methyl-L-rhamnose (15 mg.), $[\alpha]_D$ +18° (*c* 0·6), which after recrystallistaion from ether–light petroleum (b. p. 40—60°) had m. p. and mixed m. p. 95—96°, and 2,3,4,6-tetra-*O*-methyl-D-galactose (94 mg.), $[\alpha]_D$ +91° (*c* 0·97), which furnished the aniline derivative, m. p. 191—192° and mixed m. p. (with sample, m. p. 198°) 195°, and mixed fractions (17 mg.) which contained the above two sugars and a trace of an unknown sugar (R_G 1·01).

Fraction 3. The sugar was characterised as 2,3,4-tri-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 166—167°.

Fraction 5. The sugar crystallised on standing and had m. p. and mixed m. p. (with 3-*O*-methyl-L-rhamnose) 115—118°.

Fraction 12. The syrup (38 mg.) was fractionated by ionophoresis in borate buffer to give fraction 12a (8 mg.), $[\alpha]_D$ +73° (*c* 0·26), which was chromatographically and ionophoretically indistinguishable from 2,3,4-tri-*O*-methyl-D-glucose, and fraction 12b (14 mg.), $[\alpha]_D$ +23° (*c* 0·32), which was recrystallised from ether–light petroleum (b. p. 40—60°) to give 3,4-di-*O*-methyl-L-rhamnose, m. p. and mixed m. p. 93—95°.

Fraction 13. The sugar was characterised as 2,3,4-tri-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 162—163°.

Fraction 14. The sugar was recrystallised from methanol–ether to give 3-*O*-methyl-L-rhamnose, m. p. and mixed m. p. 115—117°.

Preparation and Hydrolysis of Methylated Combretum leonense *Gum*.—The gum (20 g.) was methylated successively with methyl sulphate and sodium hydroxide, and methyl iodide and silver oxide, to give methylated gum (7·5 g.) (Found: OMe, 40·8%, not raised on further methylation). The methylated gum (7 g.) was kept in 2N-sulphuric acid (200 ml.) for 3 days, the solution was diluted to 400 ml., warmed slowly so that the methylated polysaccharide remained in solution, and heated on a boiling-water bath for 8 hr. (constant rotation). The cooled solution was neutralised with barium hydroxide and barium carbonate, filtered, and concentrated to a syrup (5·7 g.) which was placed on a cellulose column (80 × 3·5 cm.). Elution of the column with butan-1-ol half saturated with water gave neutral sugars (3·4 g.), and elution with water gave barium salts of methylated acidic sugars (0·98 g.). The neutral methylated sugars were separated on cellulose (80 × 3·5 cm.), (i) light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3, later 1 : 1, and 1 : 2) saturated with water, and (ii) butan-1-ol half saturated with water being used as eluants to give 16 fractions. The barium salts were treated with Amberlite resin IR-120(H), to give the methylated acidic sugars, which were converted into the methyl ester methyl glycosides with methanolic hydrogen chloride, reduced with lithium aluminium hydride, and hydrolysed with N-sulphuric acid, to give a mixture (0·76 g.) of methylated sugars which was separated on cellulose in a similar manner to give fractions 17—24. Table 2 summarises the results of preliminary examination of the various fractions.

Fraction 1. The sugar was characterised as 2,3,5-tri-*O*-methyl-L-arabinose by conversion into 2,3,5-tri-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 137—138°.

Fraction 3. The syrup (191 mg.) was separated on filter sheets in solvent F to give 5 fractions. Fraction 3a (3 mg.) was chromatographically indistinguishable from 2,3,5-tri-*O*-methyl-L-arabinose. Fraction 3b (11 mg.), $[\alpha]_D$ +113° (*c* 0·37), was chromatographically indistinguishable from 2,3,4-tri-*O*-methyl-L-arabinose, but attempts to prepare derivatives were unsuccessful. Fraction 3c (42 mg.), $[\alpha]_D$ −23° (*c* 0·61), contained 2,5- and 3,5-di-*O*-methylarabinose and 3,4-di-*O*-methylrhamnose and gave arabinose and rhamnose on demethylation. Fraction 3d (41 mg.), $[\alpha]_D$ −6° (*c* 0·33), was characterised as 2,5-di-*O*-methyl-L-arabinose by conversion into 2,5-di-*O*-methyl-L-arabonamide, m. p. 125—127° and mixed m. p. (with sample, m. p. 127—128°) 126—127°. Fraction 3e (10 mg.) was chromatographically indistinguishable from 2,3,6-tri-*O*-methyl-D-galactose.

Fraction 4. The sugar was characterised as 2,3,6-tri-*O*-methyl-D-galactose by conversion into 2,3,6-tri-*O*-methyl-D-galactonolactone, m. p. 97—98° and mixed m. p. (with sample, m. p. 98—99°) 98—99°.

Fraction 5. A portion of the syrup was treated with methanolic hydrogen chloride, and examination of the products by gas chromatography on column *a* indicated the presence of methyl glycosides of 2,3-di-*O*-methylarabinose, 2,3,4- and 2,3,6-tri-*O*-methylgalactose in the

TABLE 2

Analysis of hydrolysate of methylated *Combretum leonense* gum *

Fraction	Wt. (mg.)	$[\alpha]_D$	R_G	Paper chromatography Sugar	Sugars given on demethylation	Other evidence
1	535	−41°	0·99	2,3,5-Me_3 arabinose		F, G
2	22		0·99	2,3,5-Me_3 arabinose		F, G
			0·93	Me_4 galactose		
3	191		0·86	2,5-Me_2 arabinose + others		
4	34	+83	0·76	2,3,6-Me_3 galactose		A, F, G
5	423	+115	0·73	2,3,6-Me_3 galactose 2,3,4-Me_3 galactose	Galactose	A, B, F, G
			0·61	2,3-Me_2 arabinose	Arabinose	
6	361	+109	0·68	2,3,4-Me_3 galactose		A, F, G
7	55	+57	0·74	2,3,4-Me_3 galactose		F, G, P
			0·62	3-Me rhamnose ? 2,6-Me_2 galactose (t)		
8	44	+27	0·68	2,3,4-Me_3 galactose (t)		F, G
			0·62	3-Me rhamnose		
9	94	+80	0·54	2,6-Me_2 galactose 2,3-Me_2 galactose (t)		F, G, I, P
10	105	+76	0·54	2,3-Me_2 galactose 2,4-Me_2 galactose	Galactose	I, P
11	337	+91	0·50	2,4-Me_2 galactose		F
12	268		0·52	2,4-Me_2 galactose		F
			0·42	Rhamnose		
13	37		0·42	Rhamnose (t)		
			0·29	2-Me galactose Unknown sugar (t)		F, G
14	99		0·29	2-Me galactose Unknown sugar (t)		F, G
15	427	+85	0·29	2-Me galactose		F
16	97		Streak	3-Me galactose Arabinose Galactose Acidic sugar		
17	17	+100	0·75	2,3,4-Me_3 galactose		F, G
18	104	+39	0·75	2,3,4-Me_3 galactose (t)		F, G, I
			0·66	2,3-Me_2 glucose 3-Me rhamnose		
19	37	+35	0·66	3-Me rhamnose		F, G
20	30	+41	0·60	3-Me rhamnose	Rhamnose	F, G, P
			0·51	2,3-Me_2 galactose	Galactose	
21	180	+85	0·52	2,3-Me_2 galactose		F, G, I, P
22	97	+56	0·51	2,3-Me_2 galactose 2,4-Me_2 galactose		F, G
			0·40	Rhamnose		
23	59	+76	0·50	Unknown sugar	Glucose (t)	F, G, P
			0·35	2-Me galactose	Galactose	

* See footnotes to Table 1.

approximate ratio of 1 : 2 : 1·5. The syrup (250 mg.) was fractionated on cellulose using light petroleum (b. p. 100—120°)–butan-1-ol (1 : 1), saturated with water, as eluant, and a chromatographically pure sample of 2,3,4-tri-*O*-methyl-D-galactose (43 mg.) was isolated and characterised as the aniline derivative, m. p. and mixed m. p. 162—164°. A chromatographically pure sample of 2,3-di-*O*-methyl-L-arabinose was obtained by separation of the mixture on paper impregnated with dimethyl sulphoxide using benzene–dimethyl sulphoxide (19 : 1) as the mobile phase, and the sugar was characterised by conversion into 2,3-di-*O*-methyl-L-arabonamide, m. p. 154—155° and mixed m. p. 153—154°.

Fraction 6. The sugar was characterised as 2,3,4-tri-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 168—169°.

Fraction 8. The syrup (44 mg.) was separated on filter sheets in solvent F, to give 2,3,4-tri-*O*-methylgalactose (6 mg.) and 3-*O*-methyl-L-rhamnose (29 mg.), $[\alpha]_D$ +27° (*c* 0·32), which, after recrystallisation from methanol–ether, had m. p. and mixed m. p. 115—117°.

Fraction 9. The main component of the syrup crystallised on seeding with 2,6-di-*O*-methyl-D-galactose. The sugar had m. p. 104—106° and was further characterised by conversion into

the aniline derivative, identified by m. p. and mixed m. p. 115—117°, and by *X*-ray powder photograph.

Fraction 10. The syrup (105 mg.) was separated on filter sheets in solvent F, to give 2,4-di-*O*-methyl-D-galactose (7 mg.), $[\alpha]_D$ +63° (*c* 0·16), isolated as the monohydrate, m. p. and mixed m. p. 89—91°, and 2,3-di-*O*-methyl-D-galactose (72 mg.), $[\alpha]_D$ +71° (*c* 0·64), which was characterised by conversion into the aniline derivative, identified by m. p. and mixed m. p. 124—126°, and by *X*-ray powder photograph.

Fraction 11. The sugar was recrystallised from ethyl acetate to give 2,4-di-*O*-methyl-D-galactose, m. p. and mixed m. p. 103°, and was further characterised as the aniline derivative, m. p. and mixed m. p. 215—216°.

Fraction 12. The mixture (268 mg.) of sugars was separated on filter sheets in solvent F, to give 2,4-di-*O*-methyl-D-galactose monohydrate (205 mg.), m. p. and mixed m. p. 90—91°, and L-rhamnose monohydrate (26 mg.), m. p. 86—88° and $[\alpha]_D$ +6° ⟶ +13° (*c* 0·63).

Fraction 15. The sugar was recrystallised from acetone–water to give 2-*O*-methyl-D-galactose, m. p. 154—155° and mixed m. p. (with sample, m. p. 157°) 155—156°.

Fraction 16. The mixture (97 mg.) of sugars was separated on filter sheets in solvent B to give fractions 16a (7 mg.), which contained galactose, 16b (12 mg.), which contained arabinose and a trace of 3-*O*-methylgalactose, 16c (13 mg.), which was chromatographically indistinguishable from 3-*O*-methyl-D-galactose and on oxidation with periodate furnished a mono-*O*-methylpentose (R_G 0·40), and 16d (10 mg.), which contained a methylated acidic sugar. Reduction of the methyl ester methyl glycoside of the acidic sugar with lithium aluminium hydride, followed by hydrolysis, gave 2,3,4-tri-*O*-methylgalactose (R_G 0·66).

Fraction 17. The sugar was characterised as 2,3,4-tri-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 168°.

Fraction 18. The syrup (104 mg.) was separated on filter sheets in solvent F, no attempt being made to recover the trace of 2,3,4-tri-*O*-methylgalactose. Fraction 18a (36 mg.), $[\alpha]_D$ +32° (*c* 0·3), crystallised on standing and had m. p. and mixed m. p. (with 3-*O*-methyl-L-rhamnose) 108—114°. Fraction 18b (21 mg.) contained a mixture of the two sugars and was not examined further. Fraction 18c (31 mg.) was characterised as 2,3-di-*O*-methyl-D-glucose by conversion into 2,3-di-*O*-methyl-D-gluconophenylhydrazide, m. p. and mixed m. p. 173—175°.

Fraction 19. The crystalline sugar was identified as 3-*O*-methyl-L-rhamnose by m. p. and mixed m. p. 108—114°, $[\alpha]_D$ +10°, +34° (equil.) (*c* 0·41), and by *X*-ray powder photograph.

Fraction 21. The sugar was characterised as 2,3-di-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. 133—135° and mixed m. p. 131—132°.

Fraction 22. The mixture was separated on filter sheets in solvent F, to give L-rhamnose monohydrate (18 mg.), m. p. 90—92°, 2,4-di-*O*-methyl-D-galactose monohydrate (14 mg.), m. p. 82—85°, and 2,3-di-*O*-methylgalactose (28 mg.) containing a trace of the 2,4-dimethyl ether.

Fractionation of Combretum leonense *Gum on Diethylaminoethylcellulose.*—The gum (300 mg.) in water (20 ml.) was poured on to a column (40 × 2 cm.) of diethylaminoethylcellulose (phosphate form) as described by Neukom *et al.*[3] The column was eluted successively with 0·025, 0·05, 0·1, and 0·25M-sodium dihydrogen phosphate (pH 6) (500 ml. each), and a gradient of sodium hydroxide (0—0·3M; 2 l.). Fractions were collected and analysed for sugars by the phenol–sulphuric acid method [6] and for uronic anhydride by the carbazole method.[7] Appropriate fractions were combined, reduced in volume, dialysed, treated with Amberlite resins IR-120(H) and IR-4B(OH), and freeze-dried. The yields of polysaccharide, estimated by the phenol–sulphuric acid method [6] and actually isolated, uronic anhydride contents (*a* by the carbazole method and *b* by decarboxylation), and optical rotations (in 0·1M-sodium hydroxide) and given in Table 3.

The products of total and partial acid hydrolysis of the various polysaccharide fractions were examined by paper chromatography in appropriate solvent systems (see Part I). Fraction I gave arabinose, galactose, and 6-*O*-galactosylgalactose. Fractions II and III each gave arabinose, galactose, rhamnose, 6-*O*-galactosylgalactose, 3-*O*-galactosylarabinose (in trace amounts), and the two aldobiouronic acids, 2-*O*-(galactosyluronic acid)rhamnose and 6-*O*-(glucosyluronic acid)galactose.

[6] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Analyt. Chem.*, 1956, **28**, 350.

[7] E A. McComb and R. McCready, *Analyt. Chem.*, 1952, **24**, 1630.

TABLE 3

Fractionation of *Combretum leonense* Gum

	Eluant	Yield (mg.) estimated	Yield (mg.) isolated	Uronic anhydride (*a*)	Uronic anhydride (*b*)	$[\alpha]_D$
Original gum ...				13·6	15·2	−15·9°
Fraction I	0·025M-NaH_2PO_4	20	20	nil	—	+12 ± 2°
Fraction II	0·25M-NaH_2PO_4	203	160	13·5	15·3	−17 ± 1
Fraction III ...	0·03M-NaOH	33	28	16·9	—	−20 ± 5

Larger quantities (2 × 2 g.) of the gum were separated in a similar manner and furnished fraction II (1·61 g.) (Found: uronic anhydride, 15·6%) and fraction III (350 mg.) (Found: uronic anhydride, 20·4%). Only small amounts (*ca.* 60 mg.) of fraction I were isolated, possibly owing to losses in dialysis. Methylation of fraction II (300 mg.) gave methylated polysaccharide (72 mg.) (Found: OMe, 40·6). Methylated fraction III was also prepared, and complete methylation was checked by the virtual absence of infrared absorption at *ca.* 3600 $cm.^{-1}$. Samples of methylated fractions II and III were heated with methanolic hydrogen chloride, and the resulting methyl glycosides were examined by gas chromatography on columns *a* and *b*. The results showed the presence of the same cleavage products from the two methylated poly-

TABLE 4

Examination of methanolysis products from methylated *Combretum leonense* gum samples

Sugar	Relative retention times (*T*) of methyl glycosides: Column *a*	Column *b*
2,3,4-Tri-*O*-methylarabinose	(1·02)	(0·84)
2,3,5-Tri-*O*-methylarabinose	0·55, 0·71	0·47 (0·60)
2,3-Di-*O*-methylarabinose	1·56 (1·78) (1·90)	0·65 (0·84)
2,5-Di-*O*-methylarabinose	(1·90)	0·71 (1·02)
2,3,4,6-Tetra-*O*-methylgalactose	(1·78)	1·52 (1·60)
2,3,4-Tri-*O*-methylgalactose	7·5	2·64, 2·92
2,3,6-Tri-*O*-methylgalactose	3·23, 4·71	(1·60) (2·22) 2·49
2,4-Di-*O*-methylgalactose	—	3·74, 4·40
3,4-Di-*O*-methylrhamnose	(1·02)	(0·60)
3-*O*-Methylrhamnose	3·61	(1·02)
2,3-Di-*O*-methylgalacturonic acid *	5·3	(2·22) 6·25, 7·4

* Present as methyl ester. Figures in parentheses indicate *T* values of components which were incompletely resolved.

saccharide samples, and Table 4 shows the relative retention times (*T*) of methyl glycosides of methylated sugars which had been characterised previously by the formation of crystalline derivatives or whose presence was indicated by other experiments.

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