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ANALYTICAL AND STRUCTURAL FEATURES OF THE GUM EXUDATE FROM *Combretum hartmannianum* SCHWEINF.*†

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ABSTRACT

The gum exudate from *Combretum hartmannianum* is water-soluble, forms very viscous solutions, and contains galactose (22%), arabinose (43%), mannose (10%), xylose (6%), rhamnose (4%), glucuronic acid (6%), 4-*O*-methylglucuronic acid (2%), and galacturonic acid (7%). The acidic components produced on hydrolysis of the gum were 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, and two saccharides that had the same chromatographic mobility, and contained mannose and galacturonic acid, and galactose and 4-*O*-methylglucuronic acid, respectively. Methylation and methanolysis of the gum indicated the presence of terminal uronic acid, rhamnose, xylose, galactose, arabinofuranose, and arabinopyranose. Controlled, acid hydrolysis indicated the presence of (1→3)-linked arabinopyranose side-chains and (1→6)-linked galactose residues. *C. hartmannianum* gum, when subjected to two Smith-degradations, yielded Polysaccharides I and II, both of which contained galactose, arabinose, and mannose. Insufficient crude gum was available for a complete structural study, but the molecule was shown to contain long, sparsely branched chains of (1→6)-linked galactose residues, to which are attached (1→3)-linked arabinose and (1→3)-linked mannose side-chains.

INTRODUCTION

In comparison with the *Acacia* genus, where extensive work has been carried out in the field of chemical taxonomy, little is known about the chemistry of gum exudates from the *Combretum* genus, other than the structural investigations carried out[2,3] on the gum from *C. leonense*. Commercial interest in gum exudates from this genus has increased recently. We now present analytical data for, and some structural features of, the gum from *C. hartmannianum* Schweinf.

*Dedicated to the memory of Professor Edward J. Bourne.
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EXPERIMENTAL AND RESULTS

Origin and purification of gum sample. — Gum from *C. hartmannianum* Schweinf. was collected in December 1970 at Umm Abdalla, Republic of the Sudan. The crude gum (42.3 g) was readily soluble in water (2.5 l), giving a clear, yellow solution which was easily filtered. After dialysis against running tap-water for 48 h, and distilled water for 24 h, the gum was obtained as the freeze-dried product (yield 83%).

Analytical methods. — The standard analytical methods described[4,5] previously were used. Paper chromatography (p.c.) was carried out on Whatman No. 1 paper with the organic phase of *A*, benzene–butan-1-ol–ethanol–water (1:5:3:3); *B*, acetic acid–ethyl acetate–formic acid–water (3:18:1:4); *C*, butan-1-ol–ethanol–water (4:1:5); *D*, conc. ammonia–butanone–water (1:200:17); *E*, butan-1-ol–ethanol–0.1M hydrochloric acid (1:10:5). Before using solvent *E*, papers were dipped in 0.3M sodium dihydrogen phosphate and allowed to dry. Sugar ratios were determined as described for *Anacardium occidentale* gum[6]. Methylation of polysaccharides was carried out by the Haworth[7] and Purdie[8] procedures. G.l.c. of mixtures of *O*-methyl sugars was carried out with a Pye Argon Chromatograph at argon flow-rates of ~ 100 ml.min^{-1} on columns of (*1*) 15% of ethylene glycol adipate polyester on 45–60 mesh Gas Chrom Z at 175°, and (*2*) 15% of butane-1,4-diol succinate polyester on 80–100 mesh Gas-Chrom P at 176°. Retention times (*T*) are given relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside.

Analytical data and homogeneity of the gum. — Electrophoresis on cellulose acetate paper, ultracentrifugation at 44,700 r.p.m., and molecular-sieve chromatography (Bio-gel A-150 m column) indicated that *C. hartmannianum* gum comprises a polymer-homologous series of molecules with no discrete components. The analytical data for the gum are shown in Table I.

Neutral sugars. — Hydrolysis of the gum (300 mg) with 0.5M sulphuric acid (15 ml) for 7.5 h at 100°, followed by p.c. (solvents *A* and *B*), indicated the presence of galactose, arabinose, rhamnose, xylose, and a component of chromatographic mobility similar to that of mannose. The presence of mannose was confirmed, after a larger scale hydrolysis (3 g), by chromatography of the resulting syrup on Whatman 3MM papers (solvent *B*); elution of the suspected mannose gave material having $[\alpha]_D$ +11° (lit. +14° for D-mannose), which afforded a phenylhydrazone having m.p. 199° alone and in admixture with D-mannose phenylhydrazone.

Acidic sugars. — Hydrolysis of the gum (300 mg) with M sulphuric acid (15 ml) for 7.5 h at 100°, followed by p.c. (solvent *E*), indicated the presence of large proportions of glucuronic acid (R_{GAL} 0.67, brown spot) and galacturonic acid (R_{GAL} 0.50, reddish-brown spot), a smaller amount of glucurono-6,3-lactone (R_{GAL} 1.50, yellow-brown spot), and a trace amount of 4-*O*-methylglucuronic acid (R_{GAL} 1.28, orange-pink spot).

Aldobiouronic acid components. — P.c. (solvent *B*) of the hydrolysate obtained with 0.5M sulphuric acid showed two spots at R_{GAL} 0.28 (minor) and 0.50 (major

TABLE I

ANALYTICAL DATA FOR *Combretum hartmannianum* GUM

Data for crude gum	
Moisture, %	11.9
Ash, %[a]	3.8
Nitrogen, %[a]	0.64
Hence, protein, % (N × 6.25)[a]	4.0
Acetyl, %[b]	0.4
Data for purified gum	
Recovery from crude gum, %	83
Moisture, %	8.3
Ash, %[a]	3.7
Nitrogen, %[a]	0.61
Hence, protein, % (N × 6.25)[a]	3.8
Methoxyl content, %[b]	0.25
$[\alpha]_D$ in distilled water, degrees[b]	−35
Intrinsic viscosity, $[\eta]$, ml/g[a]	63
Molecular weight, (MW × 10^5)[a]	6.4
Equivalent weight[b]	1173
Hence, uronic anhydride[b,c]	15.0

Sugar composition[b] after hydrolysis of purified gum	
4-*O*-Methylglucuronic acid[d]	1.5
Glucuronic acid	7.4
Galacturonic acid	6.1
Galactose	22
Arabinose	43
Rhamnose	4
Mannose	10
Xylose	6

[a]Corrected for moisture content. [b]Corrected for moisture and protein content. [c]If all acidity arises from uronic acid. [d]If all methoxyl groups located in this acid.

component). The absence of 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, which was found[2,3] in *C. leonense* gum, was indicated by the absence of a spot at R_{GAL} 0.90. Chromatography of a large-scale hydrolysate (5 g) was carried out on Whatman 3MM paper (solvent *B*), and the two acidic components were eluted in the normal way.

Fraction I (R_{GAL} 0.28) had $[\alpha]_D$ +20°, indicating a β linkage; hydrolysis gave (p.c., solvents *B* and *E*) galactose and glucuronic acid in approximately equal amounts. This fraction was indistinguishable (p.c., solvents *B* and *E*) from 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose characterized from *Anacardium occidentale* gum[9].

Fraction II (R_{GAL} 0.50) had $[\alpha]_D$ −27°. Hydrolysis, followed by p.c. (solvents *B* and *E*), indicated that this fraction was complex, and probably a mixture of two acids. Mannose and galacturonic acid were the major components, but smaller amounts of galactose and 4-*O*-methylglucuronic acid were also present. Attempts to separate the components of fraction II by p.c. in solvent *A* for 96 h and in solvent *B* for 48 h were unsuccessful. An aldobiouronic acid containing mannose and glucuronic acid ($[\alpha]_D$ −32°), found in gum exudates from *Anogeissus*[9–11] and *Virgilia*[12] species, has been characterised as 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose. Unfortunately, the comparatively small amount of gum available for this study did not permit complete characterisation of the mannose-containing acid in Fraction II.

Methylation analysis. — Methylation of the gum (239 mg) gave a product (123 mg), $[\alpha]_D$ −32° (Found: OMe, 39.1%). A portion of the product was

TABLE II

O-METHYL SUGARS OBTAINED FROM METHYLATED *C. hartmannianum* GUM

G.l.c. of methyl glycosides[a] (T)		*R_G after hydrolysis*[d]		O-*Methyl sugar*	*Relative amount*[c]
Column 1	*Column* 2	*Solvent* C	*Solvent* D		
(0.49)	(0.47)	0.96	1.04	2,3,4-Tri-*O*-methyl-L-rhamnose	2
(0.49), (0.52)	(0.47), (0.56)	0.96	1.04	2,3,4-Tri-*O*-methyl-D-xylose	2
(0.52), 0.67	(0.56), 0.71	0.96	1.04	2,3,5-Tri-*O*-methyl-L-arabinose	10
0.90	(1.01)	0.85	0.82	2,3,4-Tri-*O*-methyl-L-arabinose	3
(1.72)	(2.26)	0.85	0.82	2,4-Di-*O*-methyl-L-arabinose	4
(1.72)	1.57	0.85	0.82	2,3-Di-*O*-methyl-L-arabinose	2
1.52	1.77	0.90	0.96	2,3,4,6-Tetra-*O*-methyl-D-galactose	4
(3.02), 3.87	(3.12), 4.29	0.73	0.48	2,3,6-Tri-*O*-methyl-D-galactose	2
6.33	6.22	0.73	0.38	2,3,4-Tri-*O*-methyl-D-galactose	2
8.45	9.28	0.51	0.22	2,4-Di-*O*-methyl-D-galactose	2
11.0, 13.3, 14.5	12.5, 14.8, 15.8	0.51	0.15	2,3-Di-*O*-methyl-D-galactose	2
1.36	1.40	0.90	0.96	2,3,4,6-Tetra-*O*-methyl-D-mannose	Trace
2.72	2.93	0.73	0.69	3,4,6-Tri-*O*-methyl-D-mannose	2
3.42	3.37	0.64	0.69	2,4,6-Tri-*O*-methyl-D-mannose	2
7.42	7.52	0.51	0.22	2,6-Di-*O*-methyl-D-mannose	1
2.38, (3.02)	(2.26), (3.12)	—	—	2,3,4-Tri-*O*-methyl-D-glucuronic acid[b]	2
5.27	4.96	—	—	2,3,4-Tri-*O*-methyl-D-galacturonic acid[b]	1

[a]Figures in parenthesis indicate *T* values of components that were not completely resolved. [b]As methyl ester methyl glycoside. [c]This is an estimate, because of incomplete resolution of the majority of the components. [d]R_G values relative to 2,3,4,6-tetra-*O*-methyl-D-glucose.

methanolysed, and the mixture of methyl glycosides was examined by g.l.c.; the *O*-methyl sugars identified are shown in Table II. Hydrolysis of the methyl glycosides, followed by p.c. (solvents *C* and *D*), indicated the presence of 2-*O*-methyl-D-galactose (R_{GAL} 0.34, solvent *C*; 0.08, solvent *D*) in addition to the *O*-methyl sugars already characterised by g.l.c. The major component was 2,3,5-tri-*O*-methyl-L-arabinose. Large proportions of terminal galactose, rhamnose, xylose, and arabinopyranose residues were present, and glucuronic and galacturonic acid were also detected as end-groups.

Partial, acid hydrolysis of the gum. — Hydrolysis of the gum with 0.25M sulphuric acid for 1 h at 100° gave (p.c., solvents *A* and *B*) three disaccharides in approximately equal proportions. The pink colour of the component with R_{GAL} 0.84 (solvent *A*), 0.87 (solvent *B*), was indicative of an arabinose disaccharide, and the component was chromatographically indistinguishable from 3-*O*-β-L-arabinopyranosyl-L-arabinose. The presence of large proportions of 2,3,5-tri- and 2,4-di-*O*-methyl-L-arabinose in the methanolysate of the gum itself indicates this component to be 3-*O*-β-L-arabinopyranosyl-L-arabinose. Also present was a component with R_{GAL} 0.28 (solvent *A*), 0.24 (solvent *B*, brown spot), which was chromatographically indistinguishable from 6-*O*-β-D-galactopyranosyl-D-galactose. The third component, with R_{GAL} 0.60 (solvent *A*), 0.51 (solvent *B*), gave a pink spot and so would be expected to contain a pentose as one of its consituent monomers. Insufficient material was available to permit isolation and characterisation of this component, although the absence of 2,4,6-tri-*O*-methyl-D-galactose in a methanolysate of the gum indicates that it cannot be 3-*O*-β-D-galactopyranosyl-L-arabinose, which was found[2,3] in *C. leonense* gum.

Preparation and examination of Polysaccharide I. — Preliminary experiments established that reaction with 0.25M sodium periodate for 72 h was required for complete oxidation of *C. hartmannianum* gum. The gum (23.4 g) was dissolved in distilled water (1274 ml), and 0.5M sodium metaperiodate (1274 ml) was added. Oxidation was carried out in the dark for 72 h, after which time 8.7 mmol of periodate/g of polysaccharide had been reduced and 1.7 mmol of formic acid released. Ethylene glycol (12.7 ml) was added; after dialysis for 48 h, sodium borohydride (6.25 g) was added and the solution was left for 30 h. After dialysis for a further 48 h, the solution was made 0.5M with respect to sulphuric acid, kept for 48 h at room temperature, dialysed for a further 48 h, concentrated, filtered, and freeze-dried to give Polysaccharide I (7.8 g, 31%), $[\alpha]_D$ +16°.

Hydrolysis of I with M sulphuric acid, followed by p.c. (solvent *E*), indicated the absence of uronic acids. Hydrolysis with 0.5M sulphuric acid, followed by p.c. (solvents *A* and *B*), showed the presence of large proportions of galactose and arabinose, and a smaller amount of mannose. Rhamnose and xylose were absent. Partial, acid hydrolysis with 0.25M sulphuric acid gave two components [R_{GAL} 0.60 (solvent *A*), 0.51 (solvent *B*), pink spot; and R_{GAL} 0.26 (solvent *A*), 0.25 (solvent *B*), brown spot] in addition to the three monomers already identified.

Methylation of Polysaccharide I (245 mg) gave a product (132 mg) having

$[\alpha]_D$ −20.8° (Found: OMe, 37.1%). A portion of the product was methanolysed and the mixture of methyl glycosides was examined by g.l.c. The *O*-methyl sugars identified are shown in Table III. On hydrolysis of the methanolysate, p.c. (solvents *C* and *D*) showed the presence of a small proportion of 2-*O*-methyl-D-galactose in addition to the methyl glycosides identified by g.l.c. The main components were 2,3,4,6-tetra-, 2,3,6- and 2,3,4-tri-, and 2,3-di-*O*-methyl-D-galactose, 2,3,5-tri- and 2,4-di-*O*-methyl-L-arabinose, and 2,4,6-tri-*O*-methyl-D-mannose.

TABLE III

O-METHYL SUGARS OBTAINED FROM METHYLATED POLYSACCHARIDES I AND II

O-*Methyl sugar*	*Relative amount*	
	Polysaccharide I	*Polysaccharide II*
2,3,5-Tri-*O*-methyl-L-arabinose	3	2
2,3,4-Tri-*O*-methyl-L-arabinose	2	10
2,4-Di-*O*-methyl-L-arabinose	10	5
2,3,4,6-Tetra-*O*-methyl-D-galactose	5	7
2,3,6-Tri-*O*-methyl-D-galactose	4	4
2,3,4-Tri-*O*-methyl-D-galactose	3	3
2,4-Di-*O*-methyl-D-galactose	2	—
2,3-Di-*O*-methyl-D-galactose	4	—
2,3,4,6-Tetra-*O*-methyl-D-mannose	1	3
2,4,6-Tri-*O*-methyl-D-mannose	5	5
2,6-Di-*O*-methyl-D-mannose	2	—

Preparation and examination of Polysaccharide II. — Polysaccharide I (5.0 g) was dissolved in distilled water (135 ml), and 0.25M sodium metaperiodate (135 ml) was added. After oxidation for 72 h, 4.4 mmol of periodate/g of polysaccharide had been reduced and 1.8 mmol of formic acid/g of polysaccharide released. The solution was treated as for the Smith-degradation of the gum itself, to give Polysaccharide II (1.1 g, 22%), $[\alpha]_D$ +64.3°.

Hydrolysis with 0.5M sulphuric acid, followed by p.c. (solvents *A* and *B*), showed that arabinose was the major component, with smaller amounts of galactose and mannose. Partial, acid hydrolysis (0.25M sulphuric acid, 1 h) gave the two disaccharides formed from Polysaccharide I.

When Polysaccharide II was methylated by the Haworth method, the product could not be precipitated with light petroleum (b.p. 60–80°); a colloidal solution was obtained. The light petroleum solutions were therefore allowed to evaporate; the fine, white residue (methylated polysaccharide) which remained was dissolved in methanol, and a Purdie methylation was carried out; instead of pouring the chloroform syrup into light petroleum, the solution was evaporated to dryness, methanolic HCl (5%) was added, and methanolysis was carried out in the normal way. The *O*-methyl sugars identified by g.l.c. are shown in Table III. Hydrolysis of the methanolysate, followed by p.c. (solvents *C* and *D*), showed the absence of any di-*O*-methyl sugars or any 2-*O*-methyl-D-galactose.

Table IV shows the sugar composition of *C. hartmannianum* and its Smith-degradation products (Polysaccharides I and II).

TABLE IV

SUGAR CONTENT OF *C. hartmannianum* AND ITS DEGRADATION PRODUCTS

Polysaccharide	$[\alpha]_D$ (*degrees*)	*Gal*	*Ara*	*Man*	*Rha*	*Xyl*	*GalA*	*GlcA*
C. hartmannianum gum	−35	22	43	10	4	6	6	9
Polysaccharide I	+16	36	45	19	—	—	—	—
Polysaccharide II	+64	31	46	23	—	—	—	—

DISCUSSION

Purified gum from *C. hartmannianum* behaved during zone electrophoresis, ultracentrifugation, and molecular-sieve chromatography as a homogeneous system similar to *Acacia* exudates[14,15] and the gum exudate from *Anacardium occidentale*[9].

Arabinose was the major sugar constituent, together with a large proportion of galactose, and smaller amounts of mannose, xylose, rhamnose, glucuronic acid and its 4-*O*-methyl analogue, and galacturonic acid. At least two aldobiouronic acids, one of which was identified as 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, were formed on acid hydrolysis; it is suspected that two other aldobiouronic acids are present in admixture and contain mannose and galacturonic acid (major component), and galactose and 4-*O*-methylglucuronic acid (minor component), respectively. The amount of the acidic fraction isolable from the small amount of gum available was insufficient to allow a separation of these aldobiouronic acids. Their presence is suspected in hydrolysates of gums from other *Combretum* species; work is in progress[16] to characterize these acids from *C. obovatum* gum, which is more readily available.

Methylation analysis of *C. hartmannianum* gum indicated the presence, as end-groups, of xylose, rhamnose, glucuronic acid, and galacturonic acid in addition to large proportions of end-group arabinose (both in the pyranose and furanose form) and galactose. Also formed in substantial proportions were 2,4- and 2,3-di-*O*-methyl-L-arabinose, 2,3,6- and 2,3,4-tri- and 2,3-di-*O*-methyl-D-galactose, and 3,4,6- and 2,4,6-tri-*O*-methyl-D-mannose. The presence of 2,6-di- and 2-*O*-methyl-D-galactose and 2,6-di-*O*-methyl-D-mannose are attributed to undermethylation.

Partial, acid hydrolysis of the gum gave 3-*O*-β-L-arabinopyranosyl-L-arabinose, 6-*O*-β-D-galactopyranosyl-D-galactose, and a component having R_{GAL} 0.60 (solvent *A*) which almost certainly contains arabinose linked to galactose. Since 2,4,6-tri-*O*-methyl-D-galactose was absent from the methanolysate of the pure gum, the last disaccharide cannot be 3-*O*-β-D-galactopyranosyl-L-arabinose, which was found[2,3] in *C. leonense* gum. Insufficient material was available to allow further study of this component.

A Smith-degradation of the gum yielded Polysaccharide I, which contained only galactose, mannose, and arabinose (major component). Methylation analysis of Polysaccharide I gave, as the main components, 2,3,4-tri- and 2,4-di-*O*-methyl-L-arabinose, 2,3,4,6-tetra-, 2,3,6- and 2,3,4-tri-, and 2,3-di-*O*-methyl-D-galactose, and 2,4,6-tri-*O*-methyl-D-mannose.

Polysaccharide II, obtained by application of periodate oxidation and borohydride reduction to Polysaccharide I, also contained galactose, arabinose, and mannose. Methylation analysis of Polysaccharide II showed the main components to be end-group arabinopyranose and galactose residues. End-group arabinofuranose and mannose were also present, in addition to 2,4-di-*O*-methyl-L-arabinose, 2,3,6- and 2,3,4-tri-*O*-methyl-D-galactose, and 2,4,6-tri-*O*-methyl-D-mannose. An unusual feature of the methylation product was the absence of di-*O*-methylgalactose residues, although end-groups are plentiful.

Many questions concerning the structure of *C. hartmannianum* gum remain to be answered. However, the limited amount of gum available for study has enabled the analytical parameters to be determined, and the following, main structural features to be established.

(*1*) All uronic acid residues are end-group; glucuronic acid and its 4-*O*-methyl analogue are linked to galactose, and other mannose-containing aldobiouronic acids remain to be isolated and characterised. In *C. leonense gum*, very few uronic acid residues are present as end-groups[2,3]. The genus *Combretum* is large and complex botanically; studies at present in progress on other *Combretum* species lead us to believe that much more extensive differences in molecular structure will be found than in the *Acacia* group. (*2*) There are substantial proportions of terminal arabinofuranose and of (1→3)-linked arabinopyranose side-chains containing 3-*O*-β-L-arabinopyranosyl-L-arabinose. None of the arabinose side-chains are attached to position 3 of galactose, and this appears to be another structural difference in comparison with the features[2,3] established for *C. leonense* gum. (*3*) *C. hartmannianum* gum contains intra-chain mannose residues linked at position 3; mannose was not found in *C. leonense* gum[2,3]. (*4*) In *C. hartmannianum* gum, rhamnose residues are terminal, in contrast to *C. leonense* gum, in which most rhamnose residues were found to be intra-chain[2,3]. (*5*) The presence of 6-*O*-β-D-galactopyranosyl-D-galactose in all partial acid hydrolysates of *C. hartmannianum* gum indicates that its structure is based on (1→6)-linked galactose chains.

Because of current difficulties associated with the collection of authentic gum samples, there appears to be little possibility of extending these studies on *C. hartmannianum* gum. However, the preliminary study reported herein reveals the complexity of gum exudates from this genus, and shows that major structural differences exist in the gum polysaccharides from *C. hartmannianum* and *C. leonense*[2,3], which cannot therefore be regarded as a general model for gums of the genus *Combretum*.

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