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APPLICATION OF GAS-LIQUID CHROMATOGRAPHY TO THE STRUCTURAL INVESTIGATION OF POLYSACCHARIDES—III[1]

THE GUM OF *ACACIA KARROO* HAYNE

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Abstract—Hydrolysis products from methylated *Acacia karroo* gum have been separated by column chromatography and identified by standard procedures including the characterization of their methyl glycosides by GLC. The results conform to the general pattern of molecular structure possessed by other *Acacia* gum exudates. Examination of the acidic fragments from the hydrolysed methylated gum has given information about this distinctive feature of the *Acacia karroo* structure.

INTRODUCTION

A RECENT paper[1] compared the results obtained from GLC examination of the methanolysis products of eight methylated *Acacia* polysaccharide gums. In view of earlier work that indicated[2] that the gum exudate of *Acacia karroo* Hayne contains glucuronic acid residues linked in two different ways ($\beta 1 \rightarrow 6$ and $\alpha 1 \rightarrow 4$ to D-galactose), a feature which is shared by only a limited number of the *Acacia* gums hitherto examined,[1,3,4] a further study has been made of this polysaccharide by the methylation and hydrolysis procedure. An object of this work was to test further the validity of the technique of analysis of methylated polysaccharides, as complex as those derived from *Acacia* gums, by methanolysis and GLC of the cleavage products.

EXPERIMENTAL

General experimental conditions. Paper chromatography, on Whatman No. 1 paper, was carried out using the following solvent systems (all v/v): (*a*) butan-1-ol–EtOH–water (4:1:5, upper layer), (*b*) AcOEt–AcOH–formic acid–water (18:3:1:4), (*c*) AcOEt–pyridine–water (10:4:3). TLC was carried out on silica gel G with chf–MeOH mixtures in various proportions (generally 5:1, v/v). Paper ionophoresis was conducted for 2–4 hr at 10 V/cm in 0·1M-borate buffer at pH 9·2.[5] R_{gal} R_G and M_e refer to rates of movement relative to galactose, 2,3,4,6-tetra-O-methylglucose, and glucose respectively. GLC for analytical purposes was with a Beckman GC-2A instrument (helium carrier, 3-foot ¼ in. o.d. copper column of 14% ethylene glycol succinate polyester on 80–100 mesh Gas Chromosorb W at 155°, flame ionisation detector), for preparative work with the Wilkens Autoprep model A-700 (H carrier, 2-metre ⅜ in. o.d. Al column of 20% diethylene glycol succinate on 80–100 mesh Gas Chromosorb P, isothermally at temps from 150 to 190°). Retention times (T values) were measured relative to methyl 2,3,4,6-tetra-O-methyl-β-D-glucoside (actual retention time, ca. 4 minutes). Unless otherwise stated, solutions were concentrated at 35–40° and 20 mm in rotary evaporators, specific rotations were equilibrium values for aqueous solutions at ca. 20°, m.ps are

[1] Part II—M. Kaplan and A. M. Stephen, *Tetrahedron* **23**, 193 (1967).
[2] A. J. Charlson, J. R. Nunn and A. M. Stephen, *J. Chem. Soc.* 5987 (1955).
[3] A. M. Stephen and E. A. C. L. E. Schelpe, *S. Afr. Industrial Chemist* 12 (1964).
[4] D. M. W. Anderson and K. A. Karamalla, *J. Chem. Soc.*(*C*) 762 (1966).
[5] A. B. Foster, *J. Chem. Soc.* 982 (1953).

uncorrected, and sugars were revealed as characteristically-coloured spots using the *p*-anisidine hydrochloride reagent.[6] Compounds separated by TLC were revealed by spraying with 2N H_2SO_4 and heating the plates at 120°.

The methylated sugar fractions were weighed after being dried *in vacuo*, and identified (against standard substances) by a variety of procedures including the measurement of $[\alpha]_D$, paper chromatography in solvents *a*, *b* and *c*, TLC, demethylation with hot HBr aq,[7] periodate oxidation[8] before and after reduction with borohydride to the corresponding glycitols,[9] and by the preparation of derivatives. In all cases the sugar fractions were converted to their methyl glycosides by heating portions in 2% methanolic HCl for 6 hr (in sealed tubes at 95°) and examined by GLC using standard sugars for comparison; certain of the methyl glycosides were obtained as pure anomers by preparative GLC, and examined by TLC and by $[\alpha]_D$ and IR spectrum measurements.

Hydrolysis of methylated Acacia karroo *gum.* The sample of methylated *Acacia karroo* gum used was that submitted[1] to analysis by the methanolysis–GLC technique. TLC showed the specimen to be essentially homogeneous. The hydrolytic procedure chosen after trial of a number of different sets of conditions was to heat (at 96°) the methylated polysaccharide (5·74 g, dried at 60° *in vacuo*) in 98% formic acid (30 ml) for 15 min, add water, and evaporate to remove the bulk of the formic acid. All of the residual syrup was heated in H_2SO_4 (50 ml) at 96° for 5 hr, neutralized ($BaCO_3$), and filtered through Celite. The filtrate was evaporated and the resulting syrupy mixture of methylated sugars and sugar acids (as Ba salts) was fractionated by cellulose column chromatography.

*Chromatographic separation of methylated sugars.** The above hydrolysate was placed on a water-jacketed column of cellulose (100 × 4·5 cm), and eluted with mixtures of light petroleum (b.p. 80–100°) and water-saturated butan-1-ol (proportions of the alcohol being increased from 1:7 to 9:1 in twelve steps; total vol. 33 l.) and finally with EtOH and water. Fractions (on average 40 ml each) were collected every 2 hr, samples being screened by paper chromatography. In this way 17 sugar-containing fractions were obtained. The R_G values quoted in the ensuing description of these fractions were measured using solvent *a*; the *T* values are all for the *methyl glycosides* derived from the sugars (cf. Ref. 10).

In a parallel investigation the *T* values of the sugars listed below were determined on a second type of ester stationary phase (4-foot column of 6% diethylene glycol succinate polyester on Chromosorb P 80–100), with H carrier, the other conditions of GLC being kept the same. As was found in Part I,[10] the plot of log *T* values for the two ester phases was linear.

Fraction 1. A syrup (150 mg), $[\alpha]_D$ +21° (*c* 3·32), R_G 1·01; *T* 0·45 s, 0·75 w. The crystalline aniline derivative was homogeneous on TLC and had m.p. and mixed m.p. (with N-phenyl-2,3,4-tri-O-methyl-L-rhamnosylamine) 113·5–116°. Preparative GLC enabled the α- and β-methyl glycosides, *T* 0·45 and 0·75 respectively, to be collected from an authentic mixture of the methyl glycosides of 2,3,4-tri-O-methyl-L-rhamnose; TLC and their physical constants indicated that the collected samples had not undergone change on passage through the column at 170°.[11]

Fraction 2. A syrup (276 mg) consisting of 2,3,5-tri-O-methyl-L-arabinose, $[\alpha]_D$ −38° (*c* 2·76), R_G 1·00; *T* 0·59 s, 0·79 w. Preparative GLC at 150° of a portion of the methyl glycosides from this fraction gave the α-methyl glycoside (*T* 0·59), $[\alpha]_D$ −125° (*c* 2·69 in MeOH), and the β-methyl glycoside (*T* 0·79), $[\alpha]_D$ +140° (*c* 0·96 in MeOH), as colourless oils which were identical on TLC with the components passed through the column.

Fraction 3. A mixture (48 mg), $[\alpha]_D$ +76° (*c* 1·19), R_G 1·00 (tr), 0·90 and 0·80 (corresponding to 2,3,5-tri-O-methylarabinose, 2,3,4,6-tetra-O-methylgalactose and 2,3,4-tri-O-methylarabinose):

* Although the cellulose column is not sufficiently reproducible to warrant publication of an elution diagram, the actual numbers of the beakers involved in the collection of the sugars is as follows—fraction number followed by numbers of beakers: 1, 35–42; 2, 52–58; 3, 70–73; 4, 74–91; 5, 92–104; 6, 105–129; 7, 132–148; 8, 155–186; 9, 200–225; 10, 285–346; 11, 379–421; 12, 425–479; 13, 481–552; 14, 553–571; 15, 572–630; 16, 669–799; 17, 800 upwards.

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[9] M. Abdel-Akher, J. K. Hamilton and F. Smith, *J. Am. Chem. Soc.* **73**, 4691 (1951).
[10] A. M. Stephen, M. Kaplan, G. L. Taylor and E. C. Leisegang, *Tetrahedron* Suppl. 7, 233 (1966).
[11] A. G. McInnes, D. H. Ball, F. P. Cooper and C. T. Bishop, *J. Chromatog.* **1**, 556 (1958).

T 0·59 s, 0·79 w; 2·00; and 1·26. Applying appropriate molar response factors[10] the areas under the peaks on the GLC indicated the 3 sugars to be in the ratio 1:20:20 by wt.

Fraction 4. A similar mixture (154 mg) to that found in fraction 3; $[\alpha]_D$ +54° (*c* 3·86). The 3 sugars were in the ratio of 1:11:5 by weight. Preparative GLC at 170° of the methyl glycosides derived from a portion of fractions 3 and 4 combined gave methyl 2,3,4-tri-O-methyl-α*β*-L-arabinosides (*T* 1·26), $[\alpha]_D$ + 129° (*c* 1·62 in MeOH) and methyl 2,3,4,6-tetra-O-methyl-α*β*-D-galactosides (*T* 2·00), $[\alpha]_D$ ca. +90° (*c* 1·0 in MeOH), as colourless syrups identical on TLC with standards.

Fraction 5. A complex mixture (81 mg) of at least 5 sugars, $[\alpha]_D$ +17° (*c* 2·02). Small amounts of 2,3,5-tri-O-methylarabinose, 2,3,4,6-tetra-O-methylgalactose and 2,3,4-tri-O-methylarabinose were detected.

Fraction 6. A mixture (736 mg), $[\alpha]_D$ −31° (*c* 3·68), R_G 0·88 and 0·84, M_e 0 and 0·70: *T* 2·29 s, 4·57 w; and 1·26 w, 3·16 s. The ratio by wt of 2,5- to 3,5-di-O-methyl-L-arabinose in the mixture was found to be 3:2. Preparative GLC at 170° of the methyl glycosides from a portion of fraction 6 gave discoloured sub-fractions (i) methyl 3,5-di-O-methyl-*β*-L-arabinoside (*T* 1·27), $[\alpha]_D$ +52° (*c* 1·91 in MeOH) which afforded 3,5-di-O-methylarabinose on mild acid hydrolysis, (ii) methyl 2,5-di-O-methyl-α-L-arabinoside (*T* 2·30), $[\alpha]_D$ −100° (*c* 7·5 in MeOH) which afforded 2,5-di-O-methylarabinose on hydrolysis, (iii) methyl 3,5-di-O-methyl-α-L-arabinoside (*T* 3·21), $[\alpha]_D$ −65° (*c* 1·62 in MeOH) contaminated with the 2 earlier sub-fractions.

Fraction 7. A syrup (160 mg) consisting of 2,3,6-tri-O-methyl-D-galactose, $[\alpha]_D$ +85° (*c* 3·99), R_G 0·78; *T* 3·92 s, 4·83 w, 5·49 w, 6·21 m. GLC showed in addition the presence of ca. 1% of 2,5- and 3,5-di-O-methylarabinose.

Fraction 8. A mixture (166 mg) containing approximately equal quantities of 2,4,6-tri-O-methyl-D-galactose and 2,3-di-O-methyl-L-arabinose, $[\alpha]_D$ + 84° (*c* 3·32), R_G 0·76 and 0·70: *T* 5·09 m, 6·01 s; and 1·94 s, 2·29 w, 2·52 m.

Fraction 9. A syrup (59 mg) consisting of 2,3,4-tri-O-methyl-D-galactose, $[\alpha]_D$ +93° (*c* 1·32), R_G 0·72; *T* 9·1 sh, 9·8. The derived *N*-phenyl-2,3,4-tri-O-methyl-D-galactosylamine was homogeneous on TLC and had m.p. 161–163° and mixed m.p. (with specimen of m.p. 163–165°) 161–165°, $[\alpha]_D$ −41° → 34° (*c* 1·9 in MeOH).

Fraction 10. A syrup (288 mg) consisting of 3,4-di-O-methyl-L-arabinose, $[\alpha]_D$ + 142° (*c* 2·88), R_G 0·52, M_e 0·29; *T* 2·78. Periodate oxidation and paper chromatography of the product confirmed the identity of the sugar.

Fraction 11. A syrup (29 mg) consisting of 2,6-di-O-methyl-D-galactose, $[\alpha]_D$ + 60° (*c* 0·73), R_G 0·53, M_e 0·30; *T* 14·2 s, 17·7 w, 20·7 m, 27·3 vw. Periodate oxidation gave the products characteristic of 2,6-di-O-methylgalactose.

Fraction 12. A syrupy mixture (32 mg) consisting partly of a mixture (1:3 by wt) of 2,3- and 2,4-di-O-methyl-D-galactose, $[\alpha]_D$ + 32° (*c* 0·82), R_G 0·46, M_e 0·28: *T* 14·6, 18·5, 19·6, 25·9; 25·9 m, 30·45.

Fraction 13. A crystalline component (940 mg) comprising 2,4-di-O-methyl-D-galactose, $[\alpha]_D$ +88° (*c* 1·58), R_G 0·50; recrystallization from chf–light petroleum (b.p. 60–80°) yielded needles, m.p. 103–104·5°. The aniline deriv (homogeneous on TLC) had m.p. and mixed m.p. 212–215°, and the methyl glycosides had *T* 26·4 m, 31·4 s.

Fraction 14. A syrupy mixture (22 mg) of 2,4-di-O-methyl-D-galactose and a trace of 3-O-methyl-L-arabinose, $[\alpha]_D$ + 65° (*c* 0·56), R_G 0·50 and 0·38, M_e 0·28 and 0·74: *T* 26·0 m, 30·8 s; 4·58 w, 6·59 s, 11·1 w, 14·5 m.

Fraction 15. A mixture (25 mg) of the same components as those present in fraction 14, but in the ratio by wt of 1:3.

Fraction 16. A mixture (162 mg) of 2-O-methyl-D-galactose and some uronic acid-containing material, $[\alpha]_D$ +65° (*c* 3·25), R_G 0·33 and 0·12, from a soln of which in MeOH the neutral sugar (66 mg) crystallized. Recrystallization (MeOH–acetone) afforded 2-O-methyl-D-galactose, m.p. and mixed m.p. 150–155°; the M_e value 0·40 and periodate oxidation confirmed the structure. The mother liquors were separated on a short column of Dowex-1 (formate), elution with water giving more (30 mg) of the neutral sugar, and elution with 1N formic acid the acidic components. On methanolysis and GLC the acidic fraction gave methyl glycosides of 2,3,4-tri-O-methylglucuronic acid (methyl ester) (*T* 2·74 m, 3·72) and 2,3,6-tri-O-methylgalactose (the first peak of which overlapped with that of the *T* 3·72 component from the ester); the ratio by wt was 2:1.

Fraction 17. Eluted with water, a residue was obtained which was separated from salts by extraction with EtOH. The syrupy extract (1·60 g), R_G 0·16–0·21, on acid hydrolysis and paper chromatography showed uronic acid and the 2,3,6-tri-, 2,3,4-tri-, 2,4-di-, and 2-O-monomethyl ethers of galactose. Methanolysis of a second portion of the extract gave glycosides of the following (approximate molar proportions, found by GLC, in parentheses): 2,3,4-tri-O-methylglucuronic acid methyl ester (40); 2,3-di-O-methylglucuronic acid methyl ester (20; *T* 12·5, 13·4 sh, 17·1); 2,3,6-tri-O-methylgalactose (10); 2,3,4-tri-O-methylgalactose (2); 2,4-di-O-methylgalactose (20). The presence of 2,4,6-tri-O-methylgalactose was also indicated.

Examination[13] *of acidic fraction* 17. Part (1·18 g) of fraction 17, in water, was passed through Amberlite IR-120 (H^+) to remove Ba, concentrated, and freeze-dried. The residue was heated under reflux in 1·5% methanolic HCl for 6 hr, neutralized (Ag_2CO_3), filtered, concentrated, then saponified in aqueous 0·15N $Ba(OH)_2$ (50 ml) at 55° for 5 hr. The soln was treated with Amberlite IR-120(H^+), and then poured through Duolite A4(OH^-) resin (16–36 mesh; column 10 × 2·5 cm.) and eluted with water.

The neutral material (fraction 17a, 108 mg) thus eluted had $[\alpha]_D$ +79° (*c* 2·32 in MeOH) and showed several components on TLC. Analysis by GLC showed fraction 17a to contain methyl glycosides of 2,3,6-tri-O-methylgalactose (14 molar proportions), 2,4,6-tri-O-methylgalactose (3), 2,3,4-tri-O-methylgalactose (4), and 2,4-di-O-methylgalactose (64). The major component (R_f 0·55 on TLC) crystallized from a solution of 17a in chf–light petroleum (b.p. 60–80°), first as prisms m.p. 162° (the β-methyl glycoside of 2,4-di-O-methyl-D-galactose, *T* 26·2), then as a mixture m.p. 130–135°, $[\alpha]_D$ +70° (*c* 1·71) (22 mg, containing the β- and α-methyl glycosides *T* 26·2 and 31·0 respectively). Acid hydrolysis and paper chromatography of the mother liquors indicated a mixture of the methyl ethers of galactose as listed above (fraction 17 hydrolysate), including 2-O-methylgalactose.

Elution of the Duolite column at 4° with 1N NaOH until the eluate was Molisch-negative, removal of sodium by addition of Amberlite IR-120(H^+), and cautious evaporation yielded an acidic fraction 17b (870 mg), portions of which were hydrolysed and methanolysed to give: 2,3,4-tri-O-methylglucuronic acid (42 molar proportions), 2,3-di-O-methylglucuronic acid (33), 2,3,6-tri-O-methylgalactose (13), 2,4,6-tri-O-methylgalactose (2), 2,3,4-tri-O-methylgalactose (2), 2,4-di-O-methylgalactose (8), 2-O-methylgalactose (trace). The bulk of 17b was treated to convert acids to their Me esters, and reduced in THF (40 ml) with LAH (0·8 g). Working up of the product by the addition of wet AcOEt, evaporation, and extraction with chf gave a syrup from which residual Al was removed by passage in aqueous soln through Amberlite IR-120(H^+). The product (0·76 g) contained no free sugars (examination by paper chromatography and by TLC); part of it was methanolysed and analysed by GLC and the rest was hydrolysed by boiling in 1N H_2SO_4 for 7 hr, neutralized ($BaCO_3$), and concentrated to a syrup. The hydrolysate was separated on a cellulose column (60 × 3 cm) as described before, fractions being sampled and tested by paper chromatography and TLC. Eight fractions were obtained, all containing neutral sugars.

Fraction (i). A mixture of methyl glycosides (60 mg), acid hydrolysis of which gave a mixture of methylated sugars similar to those detected in subsequent fractions, 2,3,4-tri-O-methylglucose predominating. GLC of fraction (i) confirmed the presence of methyl 2,3,4-tri-O-methyl-αβ-D-glucosides.

Fraction (ii). A colourless syrup (180 mg), $[\alpha]_D$ +66° (*c* 3·6), R_G 0·87, homogeneous on TLC; *T* 2·99 m, 4·56 s. A portion on borohydride reduction and periodate oxidation gave the same sugar as was produced similarly from authentic 2,3,4-tri-O-methyl-D-glucose. Methylation of the sugar gave methyl 2,3,4,6-tetra-O-methyl-αβ-D-glucoside, identified by TLC and GLC, *T* 1·00 m and 1·52 s.

Fraction (iii). A mixture (145 mg) containing 2,3,4-tri-O-methylglucose and 2,3,6-tri-O-methylgalactose. GLC analysis indicated its composition by wt to be the glucose derivative (*T* 2·90 m, 4·52 s) 2 parts and the galactose deriv (*T* 3·94 s, 4·52 w, 5·54 w, 6·22 m) 3 parts.

Fraction (iv). A syrup (106 mg), $[\alpha]_D$ +98° (*c* 1·63), chromatographically identical with 2,3,6-tri-O-methyl-D-galactose, the identity being confirmed by GLC; not more than 3% of 2,4,6-tri-O-methylgalactose may also be present. Borohydride reduction of a sample gave the crystalline galactitol derivative, pure on TLC, in the form of colourless needles (from ether–light petroleum), m.p. 83–84°. The 2,3,6-tri-O-methyl-D-galactitol (=1,4,5-tri-O-methyl-L-galactitol) was oxidized completely by periodate, yielding the expected sugar.

Fraction (v). A mixture (10 mg) containing 2,3,6-tri-O-methylgalactose and 2,3,4-tri-O-methylgalactose in the ratio 3:1.

[13] G. O. Aspinall, E. L. Hirst and A. Nicolson, *J. Chem. Soc.* 1697 (1959).

Fraction (vi). A syrup (60 mg), $[\alpha]_D$ +58°(*c* 1·3), chromatographically identical (R_g 0·68) to 2,3-di-O-methylglucose. Borohydride reduction and periodate oxidation of a portion gave the same product as from reduced fraction (iv) above. Methanolysis and GLC gave the methyl glycosides of 2,3-di-O-methylglucose, *T* 12·1 w, 15·1 m, 21·8 s.

Fraction (vii). A crystalline residue (41 mg), identical to 2,4-di-O-methyl-D-galactose; *T* 26·2 m, 31·0 s.

Fraction (viii). A syrup (7 mg) chromatographically identified as 2-O-methylgalactose.

The methanolysate from reduced fraction 17b, on analysis by GLC, gave results which were in good agreement with those obtained by column separation of the hydrolysis products.

TABLE 1. PROPORTIONS OF SUGAR RESIDUES IN HYDROLYSATE OF METHYLATED *Acacia karroo* GUM

Methylated sugar	Wt (mg)	Mol %
2,3,4-Tri-O-methyl-L-rhamnose	150	3·2
2,3,5-Tri-O-methyl-L-arabinose	284	6·5
2,3,4-Tri-O-methyl-L-arabinose	150	3·4
2,3-Di-O-methyl-L-arabinose	87	2·1
2,5-Di-O-methyl-L-arabinose	443	10·8
3,5-Di-O-methyl-L-arabinose	296	7·3
3,4-Di-O-methyl-L-arabinose	288	7·2
3-O-Methyl-L-arabinose	20	0·5
2,3,4,6-Tetra-O-methyl-D-galactose	67	1·2
2,3,6-Tri-O-methyl-D-galactose	399	7·9
2,4,6-Tri-O-methyl-D-galactose	102	2·0
2,3,4-Tri-O-methyl-D-galactose	83	1·7
2,3-Di-O-methyl-D-galactose	3	—
2,4-Di-O-methyl-D-galactose	1156	24·6
2,6-Di-O-methyl-D-galactose	24	0·5
2-O-Methyl-D-galactose	112	2·5
2,3,4-Tri-O-methyl-D-glucuronic acid	559	10·5
2,3-Di-O-methyl-D-glucuronic acid	326	6·4

DISCUSSION

Summation of the quantities of individual methylated sugar residues found by hydrolysis of methylated *Acacia karroo* gum gives the results tabulated above; these estimates are the best that can be given in view of the particular difficulty in assaying the proportions of sugars in the acidic fractions. For example, the quantity of 2,3-di-O-methylglucuronic acid found by GLC assay is considerably higher than that indicated by the yield of 2,3-di-O-methylglucose obtained by reduction and subsequent working up of the hydrolysate. Comparison with the proportions of sugar residues found by direct GLC analysis of a corresponding methanolysate[1] of the methylated gum shows (i) that the presence of all the methylated sugars (including 2,3-di-O-methyl-L-arabinose) described earlier is now confirmed, (ii) that the proportions are in good agreement except that 2,3,6-tri-O-methyl-D-galactose and the two methylated uronic acid components are now found to be in larger amount, whereas there is less of 2,4-di-O-methyl-D-galactose, and (iii) that traces of 2,3-di-O-methyl-D-galactose and 3-O-methyl-L-arabinose have been found. Furthermore, one or two unidentified components, including possibly a di-O-methylrhamnose, have been detected in very small quantity. The 2-O-methyl-D-galactose, detected earlier by paper chromatography, has been authenticated by its isolation as the crystalline sugar. The ratio of total end-groups to branch-points is close to unity (mean value *ca.* 30%),

and the total amounts of the Me ethers of the four monosaccharide components agree well with the proportions of these sugars recorded as being present in the original polysaccharide.

The GLC method[13] has proved invaluable in the present work as a means of assaying the proportions of sugars in the simple mixtures arising from overlap of fractions from cellulose column chromatography, and in determining the component residues in the acid-resistant methylated biouronic acid and more complex fragments. The measurement of T values has been of use in the characterization of methylated sugars present in small amounts, or of which only small samples could be spared. Preparative GLC has afforded a number of Me glycosides as individual anomers, though the $[\alpha]_D$ values quoted (particularly those of the di-O-methylarabinofuranosides) are possibly low on account of impurities which were detected by IR spectroscopy.[14] The order in which the anomeric arabinofuranosides were eluted from the column depended upon whether $C_{(2)}$ carried a OMe or an OH substituent.[15]

The conclusions reached in earlier papers[1,2] regarding the structure of *Acacia karroo* are corroborated, the main framework of D-galactopyranose residues being branched through $C_{(3)}$ and $C_{(6)}$, with occasional attachment of another sugar residue at $C_{(4)}$. The L-rhamnopyranose units constitute (mainly) end-groups, and are probably attached to D-glucopyranuronic acid chain units at $C_{(4)}$. Most of the acid units, however, appear to be end-groups. The $1 \rightarrow 4$ and $1 \rightarrow 6$ linkages of acid to D-galactopyranose chain units are substantiated, the 4-linked galactose bearing no other sugar attachment but the 6-linked galactose carrying a second sugar at $C_{(3)}$. The L-arabinopyranose units are partially end-group, but appear more frequently to have a sugar attached at $C_{(2)}$. Some of the L-arabinofuranose units are end-groups, but most carry sugars attached at either $C_{(3)}$ or $C_{(2)}$ (or possibly $C_{(5)}$); this feature is prominent in a polysaccharide gum recently described[16] as being present in the cormsacs of *Watsonia pyramidata*.

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