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APPLICATION OF GAS–LIQUID CHROMATOGRAPHY TO THE STRUCTURAL INVESTIGATION OF POLYSACCHARIDES—IV[1]

THE GUMS OF *ACACIA PODALYRIAEFOLIA* A. CUNN. AND *ACACIA ELATA* A. CUNN.

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Abstract—Hydrolysis products of the methylated gums of *Acacia podalyriaefolia* and *Acacia elata* have been quantitatively separated by column chromatography and identified by standard techniques. The differences found between the two gums were larger than the errors expected of the analytical procedures, and on these grounds there appear to be small structural differences between them.

INTRODUCTION

GLC analysis of the methanolysis products of a number of methylated polysaccharide gums derived from the exudates of different *Acacia* species showed quantitative differences in the proportions of sugar units linked in definite ways in the polysaccharide.[2] Three of the *Acacia* gum structures appeared to be simpler than the others in some respects, for example in containing very small proportions of rhamnose and glucuronic acid. One of these, the gum of *Acacia pycnantha*, has been studied in considerable detail.[3] We proceeded to make a comparison between the structures of the exudates from the other two, viz. *A. podalyriaefolia* and *A. elata*, and wish to report upon the results of a study of their respective fully methylated derivatives.

DISCUSSION

The polysaccharide gums from *A. podalyriaefolia* and *A. elata* are both essentially arabinogalactans, containing in addition rhamnose and glucuronic acid to the extent of about 3%. The similarity between the polysaccharides is reflected in the analytical figures given in Table 1. Characteristics of the methylated polysaccharides, shown in the same table, further indicate a close structure relationship.

After acid hydrolysis of the methylated polysaccharides and separation, by cellulose column chromatography, of the methylated sugars released, a detailed comparison of the component sugar units in the two gums could be made. Table 2 indicates the proportions of methylated sugars† found by this procedure, with the figures quoted in an earlier paper as having been obtained on a semi-quantitative basis by direct GLC analysis, on a semi-micro scale, of methanolysates of the methylated gums. The correspondence of the results obtained by the two procedures is, as in the case of a

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† Authentication of the major components has been achieved through isolation of crystalline substances; where uncertainties of characterisation occur, mention is made at the appropriate place in the experimental section.

similar comparison made with the methylated gum from *Acacia karroo*,[1] good, with the exception of the constituent proportion of 2,4-di-O-methylgalactose (results high by the semi-micro GLC analysis). In general the differences found between the two gums are larger than the errors expected of the analytical procedures, and on

TABLE 1. CHARACTERISTICS OF THE POLYSACCHARIDES AND METHYLATED POLYSACCHARIDES FROM *Acacia podalyriaefolia* AND *Acacia elata* GUMS

	Polysaccharide			Methylated polysaccharide				
Gum exudate from:	$[\alpha]_D$ (in H_2O)	Equiv.	Mols. Galactose: arabinose: rhamnose.	$[\alpha]_D$ (in Chf)	OMe	C	H	Ash %
A. podalyriaefolia	+5·3°	3585	83:16:1	−44°	41·4	52·0	7·8	0·5
A. elata	+4°	6100	80:17:3	−39°	42·6	51·9	7·9	nil

TABLE 2. PROPORTIONS OF SUGAR RESIDUES IN HYDROLYSATE OF METHYLATED *Acacia podalyriaefolia* GUM AND *Acacia elata* GUM

Methylated sugar	*Acacia podalyriaefolia*			*Acacia elata*		
	Wt (mg)	Mol %	GLC Mol %*	Wt (mg)	Mol %	GLC Mol %*
2,3,4-Tri-O-methylrhamnose	26	0·7	0·5	7	0·2	tr
2,3,5-Tri-O-methylarabinose	202	6·2	11	353	9·2	8
2,5-Di-O-methylarabinose	31	1·0	—	34	1·0	—
3,4-Di-O-methylarabinose	—	—	1 ?	—	—	—
3,5-Di-O-methylarabinose	6	0·2	—	7	0·2	—
2,3,4,6-Tetra-O-methylgalactose	1033	25·7	26	1515	32·0	34
2,3,6-Tri-O-methylgalactose	196	5·2	5	90	2·0	1
2,4,6-Tri-O-methylgalactose	105	2·8	2	222	5·0	4
2,3,4-Tri-O-methylgalactose	287	7·6	7	217	4·9	5
2,3-Di-O-methylgalactose	35	1·0	—	—	—	—
2,4-Di-O-methylgalactose	1189	33·6	38	1511	36·3	43
2,6-Di-O-methylgalactose	44	1·2	tr	54	1·3	tr
2-O-Methylgalactose	340	10·3	8	183	4·7	4
4-O-Methylgalactose	47	1·5		15	0·5	
Galactose	15	0·5	—	—	—	—
2,3,4-Tri-O-methylglucuronic acid	46	1·1	2	115	2·5	—
2,3-Di-O-methylglucuronic acid	52	1·4	tr	20	0·5	—
Unknown	—	—	—	—	—	1

* M. Kaplan, M.Sc. Thesis, University of Cape Town, 1965.

these grounds there would appear to be structural differences between them. Each is very highly branched, the percentage of chain units (1 → 3, 1 → 4 and 1 → 6 linked galactose), differing in amount in the two gums, being only of the order of 12–15%.

D-Galactopyranose end-groups predominate (more in *A. elata*) over L-arabinofuranose. The branch-points are almost exclusively at $C_{(3)}$ and $C_{(6)}$ on D-galactopyranose.

The significance of the triply-branched D-galactopyranose units is not clear, as there seems to be a good case, on the grounds of branch-points exceeding end-groups as analysed, for regarding the 2-O-methyl-D-galactose as arising from partial failure to methylate exposed axial 4-hydroxyl groups. Some de-O-methylation during acid hydrolysis is also likely to have occurred.

L-Rhamnopyranose and D-glucuronic acid residues occur in small amounts as end-groups; some of the acid is linked through $C_{(4)}$ by some other sugar. In these respects the methylpentose and the sugar acid occur bound in exactly the same manner as in other *Acacia* polysaccharides where they represent a high proportion of the carbohydrate residues present.

Further corroboration of the proportions of methylated sugars released on acid hydrolysis of methylated gums from both *A. podalyriaefolia* and *A. elata* has been sought by borohydride reduction of the hydrolysates, acetylation of the mixtures of methylated alditols, and GLC using conditions similar to those used recently by other workers.[4,5] Qualitatively the results are satisfactory; quantitative analytical experiments are in progress, and show excellent promise.

Further discussion on the relationship between the structures of the polysaccharide gums of *A. podalyriaefolia* and *A. elata* will be presented when graded acid hydrolysis and Smith-degradation experiments have been completed.

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) and (*b*) AcOEt–pyridine–water (10:4:3). TLC was carried out on silica gel G with (*c*) Chf–MeOH mixtures in various proportions (generally 5:1, v/v), and with (*d*) butan-2-one–water azeotrope. Paper ionophoresis was conducted for 2–4 hr at 10 V/cm in 0·1M-borate buffer at *p*H 9·2[6]. R_{gal}, R_G and M_g refer to rates of movement relative to galactose, 2,3,4,6-tetra-O-methylglucose and glucose respectively. GLC was carried out on 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). 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 uncorrected, and sugars were revealed as characteristically-coloured spots, using the *p*-anisidine hydrochloride reagent.[7] 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* and *b*, TLC, demethylation with hot HBr aq,[8] periodate oxidation[9] before and after reduction with borohydride to the corresponding glycitols (many of them crystalline), and by NMR spectroscopy (Varian A-60 spectrometer, D_2O solutions).[10] 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 96°) and examined by GLC using standard sugars for comparison.

Hydrolysis of methylated Acacia podalyriaefolia *gum.* The sample of methylated *Acacia podalyriaefolia* gum[2] (8·27 g) was submitted to two further Purdie methylations, and the clear glass so obtained [5·82 g; $[\alpha]_D$ −44° (*c*, 3·31 in Chf). (Found: C, 52·0; H, 7·8; OMe 41·4; ash 0·5%)] was heated (at 96°) in 98% formic acid (20 ml) for 30 min. Water was added and the product, after evaporation, heated in 1N H_2SO_4 (120 ml, at 96°) for 7 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) fractionated by cellulose column chromatography.

Chromatographic separation of methylated sugars. The above hydrolysate was placed on a water-jacketed column of cellulose (105 × 5 cm), and eluted with mixtures of light petroleum (b.p. 100–120°) and water-saturated butan-1-ol (proportions of alcohol being increased from 1:7 to 6:1 in 9 steps; total volume 35 l.); with water-saturated butan-1-ol, and finally with EtOH and water. Fractions (on the average 40 ml each) were collected hourly in beakers, samples being screened by paper chromatography and by TLC using solvent (*c*). In this way 20 sugar-containing fractions* were obtained. The R_G values quoted in the ensuing description of these fractions were measured in solvent *a*; the *T* values are all for the methyl glycosides derived from the sugars (cf. Ref. 11).

Fraction 1. A syrup (65 mg), $[\alpha]_D$ +5° (*c*, 3·02), R_G 1·04, *T* 0·44 s, and 0·59 w. Quantitative GLC indicated this fraction to contain 2,3,4-tri-O-methylrhamnose (22 mg) and 2,3,5-tri-O-methylarabinose (2 mg). De-O-methylation in 48% HBr gave a series of methyl ethers of rhamnose.

Fraction 2. A syrup (395 mg) consisting of 2,3,4-tri-O-methylrhamnose (4 mg) and 2,3,5-tri-O-methylarabinose (200 mg), having $[\alpha]_D$ −24·3° (*c*, 2·34), R_G 1·04 (trace) and 1·00, *T* 0·44 vw, and 0·59 s, 0·78 w. De-O-methylation showed a series of arabinose methyl ethers and arabinose, identical (paper chromatography) with those obtained similarly from authentic 2,3,5-tri-O-methyl-L-arabinose. NMR spectroscopy indicated anomeric forms (α predominating) of 2,3,5-tri-O-methyl-L-arabinose (overlapping OMe signals, 9p).

Fraction 3. A syrup (1016 mg) having $[\alpha]_D$ +90·5° (*c*, 1·50), R_G 0·91, *T* 1·95, containing 2,3,4,6-tetra-O-methyl-D-galactose (1004 mg), showed a series of galactose methyl ethers and galactose on de-O-methylation. Reduction with sodium borohydride and recrystallization (from cyclohexane) gave 2,3,4,6-tetra-O-methyl-D-galactitol, m.p. and mixed m.p. (with a sample derived from methylated *Acacia elata* gum) 68–69°, $[\alpha]_D$ +9·1° (*c*, 1·51 in EtOH), R_f 0·47 (TLC, solvent *d*). Lit.[12] m.p. 68–70, $[\alpha]_D$ +12·5° (*c*, 2·25 in EtOH).

Fraction 4. A syrup (8 mg) consisting of equal weights of 2,3,4,6-tetra-O-methylgalactose, *T* 1·97 s, and 3,5-di-O-methylarabinose, *T* 1·35 w, 3·13 s.

Fraction 5. A syrup (23 mg) having $[\alpha]_D$ +16·7° (*c*, 0·87), R_G 0·91 and 0·88, M_g 0 and 0·75, *T* (1·95 w; 2·28 s, 4·53 w; and 1·35 w, 3·16 w), containing 2,3,4,6-tetra-O-methylgalactose (2 mg), 2,5-di-O-methylarabinose (19 mg) and 3,5-di-O-methylarabinose (2 mg). De-O-methylation gave arabinose and a series of its methyl ethers.

Fraction 6. A syrup (43 mg) having $[\alpha]_D$ +52·5° (*c*, 1·78), R_G 0·91, 0·88 and 0·83, *T* (1·97 w; 2·28 s, 4·53 w; and 3·77 w, 4·52 w, 5·17 w, 5·82 w), corresponding to 2,3,4,6-tetra-O-methylgalactose (1 mg), 2,5-di-O-methylarabinose (12 mg) and 2,3,6-tri-O-methylgalactose (2 mg). De-O-methylation showed a series of methyl ethers of arabinose and galactose and the sugars themselves.

Fraction 7. A syrup (100 mg) having $[\alpha]_D$ +85·5° (*c*, 1·20), R_G 0·78, *T* 3·76 s, 4·75 w, 5·18 w, 5·77 m, containing 2,3,6-tri-O-methylgalactose (93 mg) and 2,4,6-tri-O-methylgalactose (7 mg). De-O-methylation gave a series of methyl ethers of galactose and galactose. Recrystallization of the borohydride-reduced derivative from ether–light petroleum (b.p. 60–80°) afforded needles, m.p. and mixed m.p. (with a sample obtained from methylated *Acacia karoo* gum[1]) 86–87°, R_f 0·32 (TLC, solvent *d*). Periodate oxidation and paper chromatography of this product (2,3,6-tri-O-methyl-D-galactitol) confirmed the identity of the main sugar component.

Fraction 8. A syrup (102 mg) having $[\alpha]_D$ +92·5° (*c*, 1·28), R_G 0·78, *T* 3·73 s, 4·75 w, 5·13 w, 5·70 m and showing two spots on TLC (solvent *d*), corresponding to 2,3,6-tri-O-methylgalactose (72 mg) and 2,4,6-tri-O-methylgalactose (30 mg). A portion of fraction 8 was reduced, and oxidized with periodate: paper chromatography then confirmed the identity of 2,3,6-tri-O-methylgalactose in this fraction, and TLC showed the presence of unchanged 2,4,6-tri-O-methylgalactitol after the periodate treatment. De-O-methylation of the sugar mixture gave a similar series of methyl ethers to those found for fraction 7.

Fraction 9. A syrup (84 mg) having $[\alpha]_D$ +89° (*c*, 1·15), R_G 0·78 and 0·74, *T* (3·75 w, 4·97 m and 5·78 s, and 9·30 m), corresponding to 2,3,6-tri-O-methylgalactose (11 mg), 2,4,6-tri-O-methylgalactose (43 mg) and 2,3,4-tri-O-methylgalactose (27 mg).

Fraction 10. A crystalline component (258 mg) having $[\alpha]_D$ +102° (*c*, 2·25), R_G 0·73, *T* 5·41 vw, and 9·30 s, corresponding to 2,4,6-tri-O-methylgalactose (18 mg) and 2,3,4-tri-O-methylgalactose (216 mg). Recrystallization (from ether containing a trace of acetone) afforded needles, m.p. and mixed m.p. (with

* These were made up as follows, fraction numbers preceding the numbers of beakers used in the collection: 1, 1–18; 2, 19–90; 3, 91–130; 4, 131–137; 5, 138–150; 6, 151–176; 7, 177–203; 8, 204–220; 9, 221–237; 10, 238–276; 11, 277–362; 12, 363–381; 13, 382–391; 14, 392–413; 15, 414–563; 16, 564–590; 17, 591–601; 18, 602–637; 19, 638–657; 20, 658–upwards.

authentic 2,3,4-tri-O-methyl-α-D-galactose) 61–63°. The derived glycitol had m.p. and mixed m.p. (with a sample derived from methylated *Virgilia oroboides* gum[13]) 122–123°, (lit.[12] m.p. 122–123°), R_f 0·29 (TLC, solvent *d*). The crystalline sugar was shown by NMR spectroscopy to consist of the α form, with OMe signals corresponding to those found for an authentic specimen of 2,3,4-tri-O-methyl-D-galactose. After mutarotation $H_{(1)}\beta$ (doublet) and $C_{(2)}\beta$ OMe signals were observed.

Fraction 11. A syrup (47 mg) having $[\alpha]_D$ +137° (*c*, 1·99), R_G 0·70 and 0·78, *T* ($C_{(2)}\beta$ OMe 1·92 s; 5·08 vw, 5·66 vw; 9·43 m; and 22·75 m, 26·75 s), containing 2,3,4,6-tetra-O-methylgalactose (17 mg), 2,4,6-tri-O-methylgalactose (1 mg), 2,3,4-tri-O-methylgalactose (8 mg) and 2,4-di-O-methylgalactose (19 mg). Paper chromatography of the hydrolysate of this fraction both before and after borohydride reduction showed the above-mentioned methylated sugars. De-O-methylation of the mixture gave a series of methyl ethers of galactose including 2,3,4,6-tetra-O-methylgalactose and galactose. It appears as if the constants R_G 0·78 (not present after hydrolysis) and *T* 1·92, and 22·75, 26·75 are due to an oligosaccharide of 2,3,4,6-tetra-O-methylgalactose and 2,4-di-O-methylgalactose containing a large percentage of α linkages (see also fraction 18).

Fraction 12. A syrup (30 mg) having $[\alpha]_D$ +69° (*c*, 1·25), R_G 0·61, M_g 0·30, *T* 13·00 s, 16·10 w, 18·50 m, 24·42 vw, corresponding to 2,6-di-O-methylgalactose (27 mg). De-O-methylation and paper chromatography gave galactose and one spot corresponding to a methyl ether (2- and/or 6-). Periodate oxidation and paper chromatography gave products characteristic of a 2,6-di-O-methylhexose.

Fraction 13. A syrup (20 mg) having $[\alpha]_D$ +79° (*c*, 0·86), R_G 0·55 and 0·61, M_g 0·30, *T* 13·06 s, 15·85 w, 18·01 m, 23·70 w, was judged to be a mixture of 2,3-di-O-methylgalactose (10 mg) and 2,6-di-O-methylgalactose (10 mg). De-O-methylation and paper chromatography gave galactose and monomethyl ethers thereof.

Fraction 14. A syrup (27 mg) having $[\alpha]_D$ +102° (*c*, 1·12), R_G 0·55 and 0·61, M_g 0·30, *T* 13·77 s, 16·70 w, 18·35 m, 23·80 m. This corresponded to 2,3-di-O-methyl-D-galactose (25 mg) containing a trace of 2,6-di-O-methylgalactose (2 mg). Periodate oxidation and paper chromatography of the derived glycitols, R_f 0·14 (TLC, solvent *d*), gave the expected products.

Fraction 15. The crystalline sugar (1149 mg), $[\alpha]_D$ +86·5° (*c*, 1·56), R_G 0·55, M_g 0·27, *T* 23·30 m, 27·00 s, containing 2,4-di-O-methylgalactose (1121 mg), separated as needles from Chf–light petroleum (b.p. 60–80°), m.p. and mixed m.p. (with 2,4-di-O-methyl-α-D-galactose) 106–107°. The derived methyl 2,4-di-O-methyl-β-D-galactoside had m.p. 166–167° (lit.[1,14] m.p. 162°, 167°), *T* 24·3. Reduction of the sugar and recrystallization of the glycitol (from AcOEt) yielded needles, m.p. and mixed m.p. (with sample derived from methylated *Acacia elata* gum) 135–136°, $[\alpha]_D$ +27·8° (*c*, 2·28 in MeOH), R_f 0·16 (TLC, solvent *d*); lit.[12] m.p. 134–135°, $[\alpha]_D$ +23·5° (*c*, 2·04 in MeOH). Periodate oxidation of the glycitol gave the expected sugar (2,4-di-O-methyllyxose) on paper chromatography. The crystalline sugar was shown to be the α anomer by NMR spectroscopy, sharp OMe singlets corresponding to OMe at $C_{(2)}$ α and $C_{(4)}$ being observed; after mutarotation $H_{(1)}$ β (doublet) and $C_{(2)}$ β OMe signals appeared.

Fraction 16. The crystalline sugar (323 mg), $[\alpha]_D$ +81° (*c*, 1·11), R_G 0·38, M_g 0·34, separated from MeOH–acetone as nodules, m.p. and mixed m.p. (with 2-O-methyl-β-D-galactose) 155–156°. Periodate oxidation and paper chromatography gave the expected methoxymalondialdehyde. Reduction and recrystallization (from MeOH–AcOEt) afforded 2-O-methyl-D-galactitol, m.p 105°, $[\alpha]_D$ +4·8° (c, 1·04 in MeOH), R_f 0·06 (TLC, solvent *d*). The structure of the sugar (β form, mutarotating slowly) was confirmed by comparison of its NMR spectrum with that of an authentic sample.

Fraction 17. The mixture (51 mg) having $[\alpha]_D$ +93° (*c*, 2·52), R_G 0·38, 0·29, M_g 0·34, 0·23, was judged from paper chromatography and ionophoresis to be composed of 2-O-methylgalactose and 4-O-methylgalactose in the ratio 1:2.

Fraction 18. A partly-crystalline mixture (39 mg) having $[\alpha]_D$ +76·5° (*c*, 1·94), R_G 0·30, 0·43, M_g 0·24 and 0, was judged to be composed of 4-O-methylgalactose (1 part) and an unidentified component (2 parts). After treatment with mixed ion-exchangers and charcoal, the syrup crystallized as needles, m.p. 207° (after having been washed with acetone). Periodate oxidation and paper chromatography gave the product expected of 4-O-methyl-D-galactose. The mother liquor (R_G 0·43) was methanolysed, analysed by GLC and shown to contain 2,3,4,6-tetra-O-methylgalactose (1 mol.), 2,3,6-tri-O-methylgalactose (9), 2,3,4-tri-O-methylgalactose (2) and 2,4-di-O-methylgalactose (88). The appearance of a large proportion of 2,4-di-O-methylgalactose (confirmed by hydrolysis and paper chromatography) suggests the component R_G 0·43 is an oligosaccharide of 2,4-di-O-methylgalactose. The presence of 4-O-methyl-β-D-galactose in the original partly-crystalline fraction was indicated by the $H_{(1)}$ β and OMe signals in the NMR spectrum.

Fraction 19. A crystalline component (15 mg), R_G 0·19, chromatographically identical with galactose.

Fraction 20. A syrup eluted with aqueous EtOH was separated from salts by extraction with EtOH. The syrupy extract (256 mg), having R_G 0·10–0·20, was passed through Amberlite IR-120 (H^+) to remove Ba, concentrated and freeze dried. The residue (182 mg) was heated under reflux in 2% methanolic HCl for 8 hr, neutralized (Ag_2CO_3), filtered, concentrated, then saponified in aqueous 0·15N $Ba(OH)_2$ for 6 hr. The soln was treated with Amberlite IR-120 (H^+) and then passed through Duolite A4(OH^-) resin (16–36 mesh, column 5 × 1 cm) and eluted with water at 4°.

The neutral material (fraction 20a, 50 mg) thus eluted had $[\alpha]_D$ +56·5° (*c*, 2·50). Analysis by GLC showed fraction 20a to contain the methyl glycosides of 2,3,4,6-tetra-O-methylgalactose (9 mols.), 2,3,6-tri-O-methylgalactose (7), 2,4,6-tri-O-methylgalactose (13), 2,3,4-tri-O-methylgalactose (18), 2,6-di-O-methylgalactose (5) and 2,4-di-O-methylgalactose (48). Acid hydrolysis and paper chromatography of this fraction confirmed the identity of the above-mentioned methyl ethers of galactose and indicated the presence of 2-O-methylgalactose.

Elution of the Duolite column at 4° with 1N NaOH until the eluate gave a negative Molisch response, removal of the sodium by the addition of Amberlite IR-120 (H^+) and cautious evaporation yielded an acid fraction (20b, 140 mg), portions of which were hydrolysed and methanolysed to give 2,3,4,6-tetra-O-methylgalactose (1 mol.), 2,3,6-tri-O-methylgalactose (7), 2,4,6-tri-O-methylgalactose (4), 2,3,4-tri-O-methylgalactose (20), 2,3,4-tri-O-methylglucuronic acid (31) and 2,3-di-O-methylglucuronic acid (37).

Examination of the acidic fraction. The bulk of the acid fraction 20b (125 mg) was treated with methanolic HCl to convert acids to their Me esters, and reduced in THF (20 ml) with LAH (81 mg), working up the product by the addition of wet AcOEt, evaporation, addition of water (5 ml) and sodium citrate (107 mg) to de-complex the sugar from the aluminium; evaporation and extraction into Chf gave a syrup, which was extracted into methanol and filtered, affording a syrup A (98·2 mg). A portion of this was methanolysed and analysis by GLC showed 2,3,4,6-tetra-O-methylgalactose (1 mol.), 2,3,6-tri-O-methylgalactose (16), 2,4,6-tri-O-methylgalactose (5), 2,3,4-tri-O-methylgalactose (3), 2,3,4-tri-O-methylglucose (14) and 2,3-di-O-methylglucose (61). Paper chromatography of the hydrolysate confirmed the above-mentioned sugars.

Methanolysis and methylation of syrup A (12 mg) followed by GLC analysis showed 2,3,4,6-tetra-O-methylglucose (*T* 1·00 m, 1·52 s; 75 mols), 2,3,4,6-tetra-O-methylgalactose (*T* 2·00 w; 23) and unidentified components (2). The remainder of syrup A was hydrolysed in 1N H_2SO_4 and neutralized ($BaCO_3$), and the hydrolysate (51·4 mg), having $[\alpha]_D$ + 68·5° (*c*, 2·56), was chromatographed on Whatman No. 3MM paper in solvent (*a*) and separated into the following three fractions:

Fraction (i). A syrup (15 mg) having $[\alpha]_D$ +60° (*c*, 0·65 in MeOH), R_G 0·90, *T* (1·92 w; 2·88 m, 4·24 s; 3·74 s, 4·24 s, 5·28 w, 5·90 m; 9·52 w; and 11·60 s, 14·24 m, 21·95 w) containing 2,3,4,6-tetra-O-methylgalactose (2 mols), 2,3,4-tri-O-methylglucose (23), 2,3,6-tri-O-methylgalactose (29), 2,4,6-tri-O-methylgalactose (1), 2,3,4-tri-O-methylgalactose (3), and 2,3-di-O-methylglucose (42).

The occurrence of a large quantity of 2,3-di-O-methylglucose in this fraction, as well as in the methanolysate of the LAH reduced fraction 20b above, is consistent only with the partial de-O-methylation of 2,3,4-tri-O-methylglucose on methanolysis.

Methylation, methanolysis and GLC of fraction (i) showed 2,3,4,6-tetra-O-methylglucose (*T* 1·00 m, 1·52 s) and 2,3,4,6-tetra-O-methylgalactose (*T* 1·92 m). Periodate oxidation of the fraction (i) after borohydride reduction gave the same sugar as was produced similarly from authentic 2,3,4-tri-O-methylglucitol.

Fraction (ii). A mixture (13·2 mg) having $[\alpha]_D$ +97° (*c*, 0·49) and $[\alpha]_D$ +48·5° (*c*, 0·62 in MeOH), R_G 0·79 and 0·68, *T* (2·88 vw, 4·70 w; 3·82 s, 4·70 w, 5·34 w, 5·96 m; 9·56 w; and 11·50 m, 14·20 m, 21·30 m), containing 2,3,4-tri-O-methylglucose (1 mol.) 2,3,6-tri-O-methylgalactose (52), 2,4,6-tri-O-methylgalactose (4), 2,3,4-tri-O-methylgalactose (6) and 2,3-di-O-methylglucose (37). Methylation, methanolysis and GLC assay of fraction (ii) showed a mixture of 2,3,4,6-tetra-O-methylglucose (*T* 1·00 m, 1·52 s) and 2,3,4,6-tetra-O-methylgalactose (*T* 1·92 s). Periodate oxidation of the borohydride reduced fraction (ii) gave the expected products.

Fraction (iii). A syrup (19·2 mg) having $[\alpha]_D$ +49·2° (*c*, 0·67) and $[\alpha]_D$ +59° (*c*, 0·56 in MeOH), R_G 0·68, *T* (3·79 vw, 5·85 vw; 11·55 s, 14·20 m, 21·35 w) containing 2,3,6-tri-O-methylgalactose (1 mol.), 2,4,6-tri-O-methylgalactose (1) and 2,3-di-O-methylglucose (98).

Methylation, methanolysis and GLC assay of fraction (iii) showed 2,3,4,6-tetra-O-methylglucose (*T* 1·00 m, 1·52 s) and 2,3,4,6-tetra-O-methylgalactose (*T* 1·92 vw). Periodate oxidation of the borohydride reduced fraction (iii) gave the same product as was produced similarly from authentic 2,3-di-O-methylglucitol, together with a slower moving sugar. (The latter was found to be resistant to further periodate treatment, but could be 3,4-di-O-methylxylose from partial oxidation of the glucitol). The *T* values found for the sugar were 11·55 s, 14·20 m and 21·35 w, the shape of the three peaks differing from those found

for this sugar obtained from *Acacia karroo*,[1] where the order was w, m, s. This fraction could well be a mixture of di-O-methylglucoses, including the 2,3-derivative.

Hydrolysis of methylated Acacia elata *gum.* The sample of methylated *Acacia elata* gum[2] (7·04 g) was submitted to three further Purdie methylations and the recovered material (6·0 g), having $[\alpha]_D$ −39° (*c*, 1·30 in Chf) (Found: C, 51·9; H, 7·9; OMe, 42·6; ash 0%), hydrolysed and fractionated by cellulose column chromatography as for methylated *Acacia podalyriaefolia* gum hydrolysate.

Chromatographic separation of the methylated sugars. The above hydrolysate (5·3 g) was placed on a water-jacketed column of cellulose (105 × 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 8:1 in 10 stages; total volume 42 l.) and with EtOH and water. Fractions (on the average 40 ml each) were collected hourly and screened as previously, using known sugar standards. In this way 14 sugar-containing fractions* were obtained.

Fraction 1. A syrup (4 mg) which did not respond to *p*-anisidine HCl on paper chromatography, moved with the front on TLC. GLC of the unmethanolysed fraction 1 showed *T* 0·44 s, and 0·60 m, 0·80 w, indicating the presence of the methyl glycosides of 2,3,4-tri-O-methylrhamnose (3 mg) and 2,3,5-tri-O-methylarabinose (1 mg).

Fraction 2. A syrup (378 mg) consisting of 2,3,4-tri-O-methylrhamnose (4 mg) and 2,3,5-tri-O-methyl-L-arabinose (342 mg) having $[\alpha]_D$ −4° (*c*, 3·4), R_G 0·98, *T* 0·44 vw, and 0·59 s, 0·78 w. De-O-methylation and paper chromatography showed a series of arabinose methyl ethers and arabinose, similar to those obtained from 2,3,5-tri-O-methyl-L-arabinose.

Fraction 3. A syrup (1499 mg) having $[\alpha]_D$ +106° (*c*, 1·03), R_G 0·92 *T* (0·58 w, 0·79 vw; and 2·05 vs), containing 2,3,5-tri-O-methylarabinose (10 mg) and 2,3,4,6-tetra-O-methyl-D-galactose (1470 mg). De-O-methylation gave the same series of sugars as was obtained from fraction 3 above. Borohydride reduction and recrystallization from cyclohexane gave 2,3,4,6-tetra-O-methyl-D-galactitol, m.p. 68–69°, $[\alpha]_D$ +10·3° (*c*, 1·56 in EtOH).

Fraction 4. A syrup (35 mg) having $[\alpha]_D$ +72·3° (*c*, 0·75), R_G 0·93, *T* (2·05 s; 2·28 w, 4·5 vw; and 1·33 vw, 3·18 w), containing 2,3,4,6-tetra-O-methyl-D-galactose (30 mg), 2,5-di-O-methylarabinose (2 mg) and 3,5-di-O-methylarabinose (2 mg).

Fraction 5. A syrup (49 mg) having $[\alpha]_D$ + 20° (*c*, 0·89), R_G 0·88 and 0·93, M_G 0 and 0·76, *T* (1·97 w; 2·3 s, 4·5 w; and 1·25 vw, 3·1 m), consisting of 2,3,4,6-tetra-O-methylgalactose (5 mg), 2,5-di-O-methylarabinose (32 mg) and 3,5-di-O-methylarabinose (5 mg). Periodate oxidation and paper chromatography showed unchanged 2,5-di-O-methylarabinose.

Fraction 6. A syrup (249 mg) having $[\alpha]_D$ +69° (*c*, 0·99), R_G 0·76, *T* 3·80 s, 4·70 w, 5·1 m, 5·9 s, corresponding to 2,3,6-tri-O-methyl-D-galactose (87 mg) and 2,4,6-tri-O-methyl-D-galactose (141 mg). Periodate oxidation and paper chromatography of borohydride reduced fraction 6 gave the expected 2,3-di-O-methylthreose, while TLC revealed periodate-resistant 2,4,6-tri-O-methylgalactitol.

Fraction 7. A syrup (140 mg) having $[\alpha]_D$ +100° (*c*, 0·92), R_G 0·76 and 0·74, *T* (5·02 m, 5·9 s; and 9·5 s), consisting of 2,4,6-tri-O-methyl-D-galactose (72 mg) and 2,3,4-tri-O-methyl-D-galactose (68 mg). Periodate oxidation and paper chromatography of the borohydride-reduced mixture gave the expected 2,3,4-tri-O-methyllyxose, while TLC showed periodate-resistant 2,4,6-tri-O-methylgalactitol.

Fraction 8. A crystalline component (136 mg) having $[\alpha]_D$ +99° (*c*, 1·07), R_G 0·75, *T* (5·0 w, 5·8 w; and 9·5 s), corresponding to 2,4,6-tri-O-methyl-D-galactose (4 mg) and 2,3,4-tri-O-methyl-D-galactose (132 mg). Recrystallization (from ether containing a trace of acetone) afforded needles, m.p. and mixed m.p. (with authentic 2,3,4-tri-O-methyl-α-D-galactose) 58–59°.

Fraction 9. A syrup (32 mg) having $[\alpha]_D$ +100° (*c*, 1·55), R_G 0·77 and 0·70, *T* (1·97 m; 3·80 w, 4·96 w, 5·85 w; 9·95 w; and 24·20 m, 28·80 s), containing 2,3,4,6-tetra-O-methylgalactose (7 mg), 2,4,6-tri-O-methylgalactose (2 mg), 2,3,4-tri-O-methylgalactose (4 mg) and 2,4-di-O-methylgalactose (18 mg). Paper chromatography of the hydrolysate of fraction 9 both before and after borohydride reduction showed the above-mentioned methylated sugars, while de-O-methylation gave the same series of methyl ethers as for methylated *Acacia podalyriaefolia* hydrolysate fraction 11. There appears to be present an oligosaccharide similar to the one eluted in fraction 11 above.

Fraction 10. A syrup (72 mg) having $[\alpha]_D$ +78° (*c*, 0·81), R_G 0·60, 0·55, *T* (13·7 s, 16·6 w, 19·2 m, 25·8 vw;

* Collection of the sugars was as follows—fraction number followed by numbers of beakers: 1, 1–58; 2, 59–172; 3, 173–276; 4, 277–385; 5, 386–424; 6, 425–540; 7, 541–580; 8, 581–626; 9, 627–696; 10, 697–768; 11, 769–921; 12, 922–962; 13, 963–976; 14, 977 upwards.

and 25·8 vw, 28·8 w), corresponding to a mixture of 2,6-di-O-methylgalactose (54 mg) and 2,4-di-O-methylgalactose (3 mg). Periodate oxidation and paper chromatography of the sugars gave the expected methoxymalondialdehyde together with a trace of unchanged 2,4-di-O-methylgalactose. De-O-methylation gave galactose and the expected methyl ethers thereof.

Fraction 11. The crystalline sugar (1464 mg) having $[\alpha]_D$ +86° (*c*, 1·40), R_G 0·53, *T* 24·0 m, 28·4 s, indicating that this fraction consisted of 2,4-di-O-methyl-D-galactose. Recrystallization from Chf–light petroleum (b.p. 60–80°) gave colourless needles, m.p. and mixed m.p. (with authentic 2,4-di-O-methyl-α-D-galactose) 106–107°. The derived glycitol had m.p. 135–136°, $[\alpha]_D$ +23·5° (*c*, 1·47 in MeOH).

Fraction 12. The crystalline compound (183 mg) having $[\alpha]_D$ +73° (*c*, 0·94), R_G 0·38, separated from MeOH–acetone as nodules, having m.p. and mixed m.p. (with authentic 2-O-methyl-β-D-galactose) 154–155°. The derived glycitol had m.p. and mixed m.p. (with the sample derived from methylated *Acacia podalyriaefolia* gum above) 102–104°.

Fraction 13. A crystalline component (15 mg) having $[\alpha]_D$ +64·3° (*c*, 0·71), R_G 0·32, chromatographically identical with 4-O-methylgalactose.

Fraction 14. A syrup eluted with aqueous EtOH was separated from salts by extraction with EtOH. The syrupy extract was treated with Amberlite IR-120 (H^+), and the freeze-dried product (195 mg) methanolysed then saponified with 0·15N $Ba(OH)_2$ and treated with Amberlite IR-120 (H^+) as for methylated *A. podalyriaefolia* hydrolysate fraction 20. The concentrated solution was separated into a neutral fraction (14a, 36 mg), having $[\alpha]_D$ +71·4° (*c*, 1·8 in MeOH), and an acidic fraction (14b, 145 mg).

GLC analysis of the neutral fraction 14a indicated 2,3,4,6-tetra-O-methylgalactose (6 mols), 2,3,6-tri-O-methylgalactose (6), 2,4,6-tri-O-methylgalactose (6), 2,3,4-tri-O-methylgalactose (12) and 2,4-di-O-methylgalactose (70). Hydrolysis and paper chromatography of a portion confirmed the above-mentioned sugars and indicated the presence of 2-O-methylgalactose.

Portions of the acidic fraction 14b were methanolysed and hydrolysed and shown to be composed of 2,3,4,6-tetra-O-methylgalactose (1 mol.), 2,3,6-tri-O-methylgalactose (1), 2,4,6-tri-O-methylgalactose (1), 2,3,4-tri-O-methylgalactose (5), 2,3,4-tri-O-methylglucuronic acid (79) and 2,3-di-O-methylglucuronic acid (13).

The bulk of fraction 14b was converted to the Me esters and reduced in THF with LAH. Working up as for the acidic fraction of methylated *A. podalyriaefolia* gum hydrolysate (above) gave a Chf extracted fraction, syrup B (108 mg). GLC analysis of the methanolysate of syrup B showed 2,3,4,6-tetra-O-methylgalactose (1 mole), 2,3,6-tri-O-methylgalactose (21), 2,3,4-tri-O-methylgalactose (3), 2,3,4-tri-O-methylglucose (53) and 2,3-di-O-methylglucose (22). Paper chromatography of the hydrolysate confirmed the above-mentioned major components.

Methanolysis and methylation of a portion (24 mg) of syrup B, followed by GLC analysis showed 2,3,4,6-tetra-O-methylglucose (*T* 1·00 m, 1·52 s; 75 mols), 2,3,4,6-tetra-O-methylgalactose (*T* 2·00 w; 23) and unidentified components (2). Paper chromatography of the hydrolysate confirmed this analysis.

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REFERENCES

[1] Part III, A. M. Stephen and D. C. Vogt, *Tetrahedron* **23**, 1473 (1967).
[2] M. Kaplan and A. M. Stephen, *Ibid.* **23**, 193 (1967).
[3] E. L. Hirst and A. S. Perlin, *J. Chem. Soc.* 2622 (1954); G. O. Aspinall, E. L. Hirst and A. Nicolson, *Ibid.* 1967 (1959).
[4] J. A. Sawardeker, J. H. Sloneker and A. Jeanes, *Anal. Chem.* **37**, 1602 (1965).
[5] H. Björndal, B. Lindberg and S. Svensson, *Acta Chem. Scand.* **21**, 1801 (1967).
[6] A. B. Foster, *J. Chem. Soc.* 982 (1953).
[7] L. Hough, J. K. N. Jones and W. H. Wadman, *Ibid.* 1702 (1950).
[8] L. Hough and R. S. Theobald, *Methods Carbohydrate Chem.* **2**, 203 (1963).
[9] R. U. Lemieux and H. F. Bauer, *Canad. J. Chem.* **31**, 814 (1953).
[10] J. A. Jones, E. B. Rathbone, A. M. Stephen, K. G. R. Pachler and P. L. Wessels, *S. Afri. Medical J.* **42**, 117 (1968).

[11] A. M. Stephen, M. Kaplan, G. L. Taylor and E. C. Leisegang, *Tetrahedron* Suppl. 7, 233 (1966).
[12] C. T. Bishop, *Canad. J. Chem.* **38**, 1636 (1960).
[13] A. M. Stephen, *J. Chem. Soc.* 2030 (1962).
[14] G. G. Maher, *Advances in Carbohydrate Chem.* **10**, 233 (1955).