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

ACID HYDROLYSIS PRODUCTS OF THE GUMS OF *ACACIA PODALYRIAEFOLIA, A. CUNN* AND *ACACIA ELATA A. CUNN.*

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Abstract— Partial acid hydrolysis of the gums from stems of *Acacia podalyriaefolia* and *A. elata* gives polymer mixtures, together with oligosaccharides and monosaccharides, which have been characterized by paper chromatography and by GLC of their derived glycitol acetates. The acid-degraded polymers have been shown by methylation, methanolysis and GLC assay to consist largely of galactopyranose units, present approximately equally as end-groups, 3,6-linked branch points, and chain units (3- and 6-linked). Their mol wt distribution patterns, like those of the original gums, have been shown to differ by molecular-sieve chromatography.

INTRODUCTION

A CLOSE structural similarity is apparent between the plant gums from *A. podalyriaefolia* and *A. elata*, and the proportions of sugar residues present, linked in definite ways, are nearly identical.[1,2] In order to compare the galactose frameworks of the two polysaccharides, to which the minor component sugars, arabinose, rhamnose and glucuronic acid, are attached, each has been submitted to controlled acid hydrolysis, and the fragments have been examined by chromatographic procedures.

RESULTS AND DISCUSSION

Some difference between the gum exudates of *Acacia podalyriaefolia* and *A. elata* is apparent from their respective molecular size distributions, measured by molecular-sieve chromatography (gel filtration) of specimens prepared by ethanol precipitation of aqueous solutions. As reported recently[3] the gum of *A. podalyriaefolia* falls within a relatively narrow mol wt range, the elution pattern (carbohydrate content plotted against elution volume) having a single maximum corresponding to $\bar{M}_w$ (weight-average mol wt) of 31,500. *A. elata* gum, on the other hand, showed a broad elution pattern on chromatography on Biogel P-300, with an inflection at mol wt 45,700 and three overlapping peaks in the range 29,900 to 16,800; this elution behaviour was reproduced exactly on chromatographing another sample. $\bar{M}_w$, calculated from relative peak areas, was 22,000.[4] These *Acacia* gums are of relatively low mol wt, and give aqueous solutions of low viscosity.

Partial acid hydrolysis of *A. podalyriaefolia* gum in two stages (for 15 and 35 hr) gave products which, after separation into fractions soluble and insoluble in aqueous ethanol, were examined using molecular-sieve chromatography, analysis for sugar content, and methylation analysis involving GLC. Constituent neutral mono-

saccharides were proved to be L-rhamnose, L-arabinose and D-galactose by isolation of these sugars after extended acid hydrolysis.

At each stage of the partial acid hydrolysis procedure aqueous ethanol-soluble syrups (A and A′; Table 1) were obtained and shown to contain sugars of the same mobility on paper chromatography as arabinose, rhamnose, galactose, 6-O-β-D-galactopyranosyl-D-galactose (I), 3-O-β-D-galactopyranosyl-D-galactose (II), and the related β1 → 3-linked trisaccharide (III). The corresponding precipitates A and A′ had increased galactose content compared with the other neutral sugar components, A′ containing only ca. 5% of other carbohydrate (Table 2). A′, comprising 60% of the

TABLE 1. PAPER CHROMATOGRAPHY OF SUGARS RELEASED ON HYDROLYSIS OF *Acacia podalyriaefolia* AND *A. elata* GUMS

Standard sugars	R_{gal}, solvent *a*	Syrup A	Syrup A′	Syrup B
L-Rhamnose	2·40	+	tr	—
L-Arabinose	1·44	+ + +	+ + +	+ + +
D-Galactose	1·00	+	+	+ + +
Galactobiose II	0·46	tr	tr	tr
Galactobiose I	0·28	tr	tr	tr
Galactotriose III	0·14	tr	tr	tr

Identifications confirmed by paper chromatography using solvents *b*, *c* and *d*.

TABLE 2. DEGRADED POLYSACCHARIDES FROM THE GUMS OF *A. podalyriaefolia* AND *A. elata*

Gum from	Degraded gum	$[\alpha]_D$ (in H_2O)	Gal	Ar	Rh	GlA	$\overline{M}_w$	Methylated deriv., $[\alpha]_D$ (in $CHCl_3$)
A. podalyriaefolia	A	+13°	85	9	0·5	5·5	—	−12°
	A′	+26°	93·5	1·5	—	5	18,000	−23°
A. elata	B	+26°	*				17,000	−24°

* Paper chromatography of hydrolysed B showed galactose and traces only of other sugars.

total carbohydrate in the original gum, was shown by molecular-sieve chromatography to contain material distributed over the molecular weight range 31,000 to 8000 ($\overline{M}_w$ 18,000), but with three peaks in the elution diagram.[3] Repetition of the partial hydrolysis process in a single step was followed by chromatography of the entire hydrolysate on Biogel P-300; in this experiment there was even less extensive breakdown of the gum, $\overline{M}_w$ being 24,000 despite the presence of all cleavage products including oligo- and monosaccharides.

The methylated derivatives of A and A′, analysed by methanolysis and GLC,[5] are made up of methylated sugar residues in similar proportions though with less arabinose in A′ (Table 3); A′ accordingly is a mixture containing highly branched galactan molecular frameworks (from ca. 50 to 150 sugar units) with one-third each of galactopyranose end-groups, branch points of galactopyranose linked 1 → 3 and

TABLE 3. CHROMATOGRAPHY OF HYDROLYSIS AND METHANOLYSIS PRODUCTS OF METHYLATED DEGRADED GUMS A, A AND B

Methylated sugar	R_G, solvent *a*	GLC, mol%		
		A	A′	B
2,3,5-Tri-O-methyl-L-arabinose	1·00	5	0·5	—
2,3,4,6-Tetra-O-methyl-D-galactose	0·92	32	34	31
2,3,6-Tri-O-methyl-D-galactose	0·77	1	tr	1
2,4,6-Tri-O-methyl-D-galactose	0·77	12	20	23
2,3,4-Tri-O-methyl-D-galactose	0·74	15	11	10
2,6-Di-O-methyl-D-galactose	0·60	2	—	4
2,4-Di-O-methyl-D-galactose	0·55	31	32	29
2-O-Methyl-D-galactose	0·37	*		*
4-O-Methyl-D-galactose	0·29	*		*
2,3,4-Tri-O-methyl-D-glucuronic acid	0·10–0·20	1	1·5	—
Unknown	—	1	1	2

* Traces shown on paper chromatography.

1 → 6, and galactopyranose chain units linked 1 → 3 (mainly) or 1 → 6. Specific rotations indicate predominant if not exclusive β-D-linkages.

Partial acid hydrolysis of *A. elata* gum was carried out for 24 hr in one step, and both the entire hydrolysate and the ethanol-precipitated portion thereof (B) were chromatographed separately on Biogel P-300. B was heterogeneous, having $\overline{M}_w$ 17,000 and exhibiting five peaks in the elution diagram down to a molecular weight of 5000, more completely degraded material having remained dissolved in aqueous ethanol. The complexity of the entire hydrolysate was further demonstrated by chromatography on Biogel P-10, six elution peaks appearing below 5500. $\overline{M}_w$ was calculated to be 9000.

Table 2 shows a close correlation in specific rotation, sugar composition and $\overline{M}_w$ of acid-degraded *A. elata* (B) and *A. podalyriaefolia* (A′) gums, despite their preparation under conditions that were not identical, and Tables 2 and 3 indicate that their methylated derivatives are likewise very similar—indistinguishable, in fact, by the analytical method used. The nature of the branched galactan framework, or core,[6] of these two Acacia gums is therefore typical of the genus. More detail on the distribution of the 1 → 3 and 1 → 6 linkages is emerging from Smith degradation studies. The structural chemistry of fractions obtained from the partially-hydrolysed gums will shortly be investigated.

EXPERIMENTAL

General experimental conditions were as given in Part IV,[1] but with the following solvent systems (all v/v) used in paper chromatography: (*a*) butan-1-ol–EtOH–water (4:1:5, upper layer), (*b*) AcOEt–pyridine–water (10:4:3), (*c*) butan-1-ol–AcOH–water (2:1:1), (*d*) butan-1-ol–EtOH–water (1:1:1) and (*e*) butanone–AcOH–saturated aqueous boric acid (9:1:1). Molecular-sieve chromatography was carried out on the polyacrylamide gels Biogel P-300 and P-10, packed into polythene tubes (1·25 × 50 cm) or a Sephadex laboratory column (1·5 × 90 cm). Each sample (2–5 mg polysaccharide in 1 ml M NaCl) was eluted with M NaCl,[4,7] 1 ml fractions being collected and assayed for carbohydrate.[8] Mol wts corresponding to peaks in the elution curves were found from calibration curves, relating peak elution volume to log

$\bar{M}_w$, obtained by chromatographing glucose, raffinose and dextrans[9] of known $\bar{M}_w$ on the same columns. For each gel this curve was linear over a certain mol wt range, viz. 5000 to 100,000 for P-300 and 250–15,000 for P-10; these fractionation ranges are not the same as those quoted for proteins by the manufacturers.[10]

GLC analysis of glycitol acetates was performed on a Beckman GC-2A instrument (He carrier, 3-foot ¼ in o.d. copper column of 3% ECNSS-M on 100–120 mesh Gas-Chrom Q* at 175°, flame ionisation detector); retention times (T) are given relative to hexa-O-acetyl-D-mannitol.

Molecular weight determinations of A. podalyriaefolia *gum.* The sample used was from the EtOH-precipitated material described earlier.[1,2] Sedimentation and diffusion coefficients were found[11] to be $3{\cdot}68 \times 10^{-13}$ sec and $7{\cdot}81 \times 10^{-7}$ cm^2 sec^{-1}, substitution into Svedberg's equation[12] giving $\bar{M}_w$ 33500. A value of 31,500 was found by molecular-sieve chromatography, with no material of mol wt <25,500.[3]

Neutral monosaccharide components of A. podalyriaefolia *gum*

The polysaccharide (1·2 g) was heated in N H_2SO_4 (50 ml) for 10 hr at 96°, and the cooled soln was neutralized ($BaCO_3$), filtered, passed through a column of Amberlite IR-120(H^+) resin, and concentrated to a syrup (1·1 g). Chromatography on Whatman No. 3MM papers using solvent mixture (*a*) gave the following:

Fraction 1. A crystalline sugar (600 mg), $[\alpha]_D$ +80° (*c* 1·47), m.p. 164–165°, and R_{gal} 1·00. The derived acetylated glycitol had the same retention time (T 1·15) as authentic hexa-O-acetylgalactitol.

Fraction 2. A crystalline sugar (90 mg), $[\alpha]_D$ +90° (*c* 0·94), m.p. 154–155°, having the same mobility on paper chromatography as arabinose. The derived acetylated glycitol had T 0·38, the same as that of authentic penta-O-acetyl-L-arabinitol.

Fraction 3. A syrup (24 mg), $[\alpha]_D$ +5° (*c* 1·20), identical with rhamnose on paper chromatography. The derived glycitol acetate had T 0·22, the same as that of authentic penta-O-acetyl-L-rhamnitol.

Partial acid hydrolyses of A. podalyriaefolia *gum*

(i) *Preparation and examination of products A. A. podalyriaefolia* gum (1·60 g) in 0·01N H_2SO_4 was heated for 15 hr at 96°. The cooled soln was neutralized ($BaCO_3$), filtered and concentrated. Addition of ethanol (ca. 4 volumes) caused precipitation of degraded gum A, which after being washed with EtOH, acetone and ether and dried was obtained as a white powder (1·36 g), $[\alpha]_D$ +13° (*c* 2·79). (Found: Equiv., by titration, 3170. Sugars, assayed[11,13] as glycitol acetates: galactose, 85; arabinose, 9; rhamnose 0·5%). Degraded gum A (108 mg) was methylated by the methods of Haworth (one stage, with multiple additions of methyl sulphate and sodium hydroxide) and Purdie (3 treatments) to give a product (80 mg), $[\alpha]_D$ −12° (*c* 1·00 in $CHCl_3$), with no OH groups according to its IR spectrum. Methanolysis of a portion of methylated degraded gum A and GLC examination of the resulting methyl glycosides gave the results shown in Table 3. Paper chromatography (solvent *a*) of the acid-hydrolysed glycoside mixture indicated traces of 2- and 4-O-methylgalactose in addition to the methylated sugars expected from the GLC assay.

The aqueous EtOH-soluble products of partial acid hydrolysis, together with all washings, were concentrated to a syrup A (238 mg) $[\alpha]_D$ +64° (*c* 2·16). Paper chromatography using solvents (*a*), (*b*), (*c*) and (*d*) showed syrup A to be a mixture of at least six components, a comparison of which with standard sugars is set out in Table 1.

(ii) *Preparation and examination of products A'.* Degraded gum A (1·00 g) was further hydrolysed in 0·01N H_2SO_4 (45 ml) for 35 hr at 96°, neutralized, and fractionated as previously yielding insoluble degraded gum A' (718 mg), $[\alpha]_D$ +26° (*c* 2·58). (Found: Equiv., 3540. Galactose, 93·5; arabinose, 1·5%). Soluble syrup A' (197 mg) had $[\alpha]_D$ + 64° (*c* 2·19), and its composition is indicated in Table 1.

Degraded gum A' (54 mg) in water (7 ml) was reduced with $NaBH_4$ (100 mg) during 48 hr. Excess of borohydride was decomposed on the addition of Amberlite IR-120 (H^+) resin, and borate was removed by evaporation with MeOH. Paper chromatography of an hydrolysate of the residue, using solvent (*e*), showed galactose and a trace of arabinose but neither galactitol nor arabinitol. Degraded gum A' (23 mg), hydrolysed in 0·5N H_2SO_4 (2 ml) for 1 hr on a boiling water bath, cooled, neutralized ($BaCO_3$), filtered and concentrated, yielded galactose, with traces only of arabinose and oligosaccharides I, II and III (paper chromatography using solvents *a* and *b*). Methylation of degraded gum A (140 mg) as before gave a product (150 mg), $[\alpha]_D$ −23° (*c* 2·27 in $CHCl_3$); methanolysis and GLC examination of the resulting methyl glycosides gave the quantities shown in Table 3. Paper chromatography of an hydrolysate of methylated degraded gum A', using solvent (*a*), confirmed the presence of the major constituents, and showed the absence of both 2- and 4-O-methylgalactose.

* Column packing material obtained from Applied Science Laboratories, State College, Pennsylvania.

The sedimentation and diffusion coefficients[11] of degraded gum A', $2{\cdot}87 \times 10^{-13}$ sec and $10{\cdot}94 \times 10^{-7}$ $cm^2\ sec^{-1}$ respectively, indicated a value of 18,650 for $\bar{M}_w$. Molecular-sieve chromatography[3] on Biogel P-300 gave an elution pattern with broad overlapping peaks at mol wts 26,500 (35% by weight), 18,000 (15) and 8200 (35) and an inflection at 16,500 (15) ($\bar{M}_w$ 18,000).

Hydrolysis of *A. podalyriaefolia* gum in 0·01N H_2SO_4 for 50 hr at 96°, neutralization ($BaCO_3$), filtration and concentration gave an aqueous soln, a sample of which was chromatographed on Biogel P-300. The elution curve showed a large peak (76% of total material) at mol wt 28,000, and others at 17,500 (5), 13,500 (4), 11,000 (5), 9000 (5), 7500 (2), 5200 (2) and 3700 (1) ($\bar{M}_w$ 24,000).

Molecular weight determination of A. elata *gum.* Some properties of this polysaccharide have been noted.[1,2] Sedimentation and diffusion coefficients of $3{\cdot}32 \times 10^{-13}$ sec and $8{\cdot}00 \times 10^{-7}$ $cm^2\ sec^{-1}$ gave a value of 29,500 for $\bar{M}_w$. Chromatography on Biogel P-300 gave an elution diagram with an inflection at mol wt 45,700 (5%) and peaks at 29,900 (19), 20,000 (35) and 16,800 (41), indicating $\bar{M}_w$ 22,000.

Partial acid hydrolysis of A. elata *gum*

A. elata gum (100 mg) was heated in 0·01N H_2SO_4 (40 ml) for 24 hr at 96°. The neutralized ($BaCO_3$) hydrolysate was fractionated as previously, yielding insoluble degraded gum B (51 mg), $[\alpha]_D$ +26° (*c* 0·72), which revealed galactose and negligible other sugars on hydrolysis and paper chromatography. Soluble syrup B (28 mg) was shown by paper chromatography to contain the components indicated in Table 1. Methylation as before of degraded gum B (36 mg) gave a product, $[\alpha]_D$ −24° (*c* 0·95 in $CHCl_3$), which was fully methylated according to its IR spectrum. Analysis by methanolysis and GLC gave the results in Table 3. Hydrolysis and paper chromatography indicated traces of 2- and 4-O-methylgalactose.

Molecular-sieve chromatography of the entire partial acid hydrolysate on Biogel P-300 gave an elution pattern with a small peak at mol wt 35,500 (3%), large peaks at 19,000 (40) and 5400 (31), small peaks at 4000 (16), 2800 (6) and 1800 or below (4). On Biogel P-10 an elution curve resulted having large peaks at 15,000 or above (32%) and at 5300 (37), smaller peaks at 4000 (11), 2800 (7), 1800 (6), 360 (4) and 250 or below (3). A sample of the ethanol-precipitated degraded gum B on Biogel P-300 gave an elution curve with a small peak at 34,000 (3%), large peaks at 21,000 (42) and 17,000 (32), small peaks at 7800 (10) and 5200 (13). All material of molecular weight <5000 (about 30% by weight of the total, from relative peak areas) was absent from degraded gum B.

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