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STUDIES OF THE MOLECULAR CORE OF *Grevillea robusta* GUM*

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ABSTRACT

Partial acid hydrolysis of *Grevillea robusta* gum, which removed most of the L-arabinose residues (~44% of the total carbohydrate), yielded a polysaccharide (*A*) containing galactose, arabinose, mannose, and uronic acid in the molar ratios 3:1:1:2. Smith degradation of *A* gave SD*A*, $\overline{M}_w$ 4400, which contained galactose and mannose residues in the ratio ~1:1. Few uronic acid residues survived, but SD*A* contained ~20% of erythronic acid, acetal-linked through glycolaldehyde, arising by incomplete detachment of originally 4-linked glucuronic acid residues. Prolonged exposure of SD*A* to acid caused breakdown into small fragments (mol. wts. ~1250, 700, 400, and 250) with the release of glycolaldehyde. This second stage of depolymerisation was greatly accelerated by prior carboxyl-reduction. Methylation analyses of these fragments, SD*A*, and the products obtained by carboxylate-reduction and by base-catalysed degradation of methylated SD*A* enabled conclusions to be drawn regarding the location of the Gal*p*, Man*p*, and Glc*p*A residues in the polysaccharide core.

INTRODUCTION

Re-examination of the gum polysaccharides from three species of *Prunus* (family Rosaceae, order Rosales) and more detailed investigation of that from *P. armeniaca* (apricot tree)[1] emphasised the importance of the D-mannose residues in the structure of the cores, despite their low proportions (<10%). Since some gums from the family Proteaceae (order Proteales), *viz.* those from *Hakea acicularis*[2], *Brabeium stellatifolium*[3,4], and *Grevillea robusta*[5], contain a similar proportion of D-Man*p*, investigation of the role of this sugar in their molecular structure seemed appropriate. That from *Grevillea robusta*, one of several gums from *Grevillea* species, which, by virtue of their solubility, viscosity, and relatively high resistance to hydrolysis, may have industrial applications[6], was selected for further study. An earlier investigation[5] demonstrated that the Man*p* residues were concentrated in a

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periodate-resistant core, which remained after three successive Smith-degradations. In the present study, exposure of the polysaccharide core to Smith degradation has been expedited by prior removal of peripheral L-arabinosyl units, a procedure successfully adopted in analogous studies of the gums of *Prunus armeniaca*[1] and several species of *Acacia*[7-10].

EXPERIMENTAL

Origin and purification of gum sample. — The polysaccharide studied was a further sample of the purified material obtained[5] from a specimen of the gum of *Grevillea robusta* A. Cunn. collected near Stellenbosch (Cape Province, South Africa).

General methods. — P.c. was performed with *A*, 8:2:1 ethyl acetate–pyridine–water; *B*, 10:4:3 ethyl acetate–pyridine–water; *C*, 2:1:1 1-butanol–acetic acid–water; and *D*, upper phase of 4:1:5 1-butanol–ethanol–water. *p*-Anisidine hydrochloride and ammoniacal silver nitrate were used to detect sugars and polyols. T.l.c. was performed on silica gel 60 F_{254} (0.2-mm layer; Merck), using *E*, 20:20:7 chloroform–methanol–water, and detection with anisaldehyde–H_2SO_4. G.l.c. and g.l.c–m.s. were performed[1,9] using *1*, a column (2 m × 3 mm i.d.) packed with 3% of OV-225 on Supelcoport (100–120 mesh); *2*, a glass capillary column (44 m × 0.35 mm i.d.) coated with OV-225; *3*, a quartz capillary column (30 m × 0.32 mm i.d.) with OV-225 (0.25-μm thickness) as the bonded phase (Durabond DB-225; J & W Scientific); *4*, a column (2 m × 3 mm i.d.) packed with 3% of OV-17 on Chromosorb W-HP (80–100 mesh). Neutral sugars in hydrolysates were analysed as the derived alditol acetates[11] by g.l.c. on columns *1* – *3* at 215°, or as the acetylated aldononitriles[12] on column *4* at 220°; methylated sugars were determined as the alditol acetates on column *2* at 195° or column *3* at 215°; and mixtures containing acetylated polyols as well as alditol acetates were determined on column *2* with the following temperature programme, 170° for 5 min, 170→200° at 3°/min, 200° for 5 min. In methylation analyses, effective carbon response factors[13] were used in quantification, otherwise molar response factors were determined, immediately before and after the analyses, by injection of appropriate standards. Hydrolysis was carried out under conditions (2M trifluoroacetic acid, 100°, 18 h, under nitrogen in sealed tubes) that caused ~25% and ~16% degradation of Ara and Man, respectively. These factors were applied[9] as corrections to the analytical results.

Molecular weights were determined[14] by steric-exclusion chromatography (s.e.c.) on Sepharose 4B, Bio-Gel P-10, or Bio-Gel P-2, with M sodium chloride as the eluent. Fractionations on a preparative scale were performed on a column (55 cm × 3.5 cm i.d.) of Sephadex G-10 by elution with water, or, for products having low molecular weight, on a column (70 cm × 2.5 cm) of Trisacryl GF05 by elution with 0.1M pyridinium acetate buffer (pH 5.0).

Specific colorimetric methods were used in the determinations of total carbohydrate[15], uronic acid[16], glycolaldehyde[17], and polyol[18]. Methylation by a

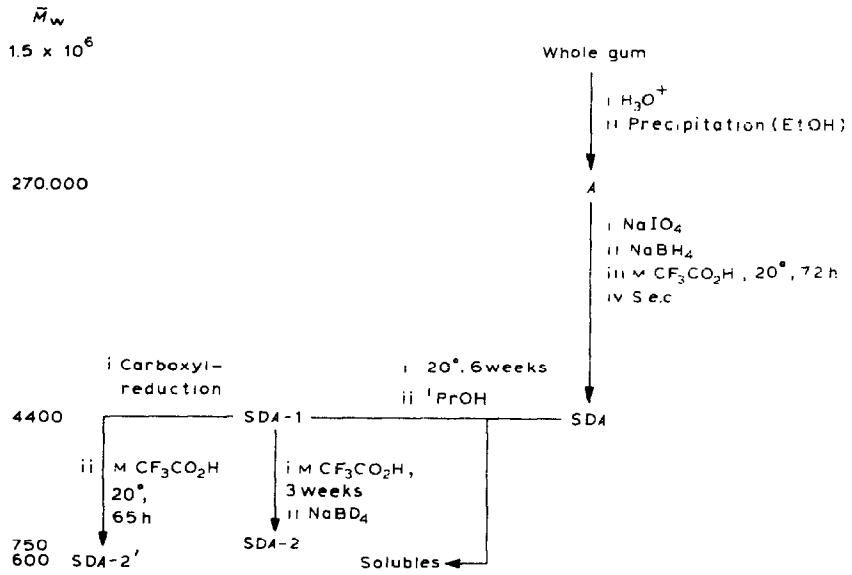
modification[19] of the Hakomori procedure[20,21] was followed by two or more treatments by the Purdie[22] method, and methoxycarbonyl groups were reduced[23] with lithium aluminium deuteride. Hydrolysates of methylated products were examined by p.c. (solvent *D*) before reduction, acetylation, and g.l.c.–m.s.

Partial hydrolysis with acid. — The purified gum polysaccharide (12 g) was treated with 5mM H_2SO_4 (500 mL) at 100° for 12 h, during which $[\alpha]_D$ changed from −8 to +57°. After centrifugation to remove an insoluble residue (2.2 g), the supernatant solution was neutralised ($BaCO_3$) and concentrated (by freeze-drying) to 250 mL. The partially hydrolysed polysaccharide (*A*, 3.4 g) was then precipitated by the addition of EtOH (4 vol.) and isolated by freeze-drying, after removal of cations with Amberlite IR-120 (H^+) resin, of an aqueous solution of the precipitate. The above insoluble residue contained Gal, Ara, Man, Xyl, and Rha in molar proportions similar to those in *A*.

The supernatant solution remaining after the addition of EtOH was de-ionised (Amberlite MB-1) and concentrated to give a syrup (3.5 g) which contained (p.c., solvent *A*) mainly Ara; some Gal and traces of Xyl, Rha, and neutral oligosaccharides having the mobilities of L-Ara*p*-(1→3)-L-Ara, β-D-Gal*p*-(1→3)-D-Gal, β-D-Gal*p*-(1→6)-D-Gal, D-galactotrioses, and D-galactotetraoses were also detected (p.c., solvent *B*), but no acidic components (p.c., solvent *C*). G.l.c. of the derivatised products of hydrolysis indicated the molar ratio of Ara and Gal to be ~9:2.

Smith degradation of A. — Oxidation of *A* (2.4 g) in 0.12M $NaIO_4$ (300 mL) for 96 h (periodate consumption[24], 5.5 mmol. g^{-1}), followed by reduction ($NaBH_4$), and treatment of the product with M trifluoroacetic acid at room temperature (~20°) for 72 h was monitored[25] by s.e.c. Fractionation of the products on Sephadex G-10 yielded SD*A* (335 mg), a mixture (350 mg) of SD*A* and inorganic salts, and a low-molecular-weight fraction (syrup, ~1 g, obtained after de-ionisation and concentration; consisting almost entirely of glycerol, with traces of erythritol, threitol, Ara, Man, and Gal). SD*A* had $[\alpha]_D$ +70°, $\overline{M}_w$ 4400 (see Scheme 1), and contained ~6 mol% of uronic acid; a trace of a component[5] having the mobility in p.c. (solvent *C*) of β-D-Glc*p*A-(1→2)-D-Man was detected in a hydrolysate. G.l.c. of the products of hydrolysis after reduction ($NaBH_4$) and acetylation showed Gal and Man (molar ratio ~1:1) to be the only neutral sugar components, together with glycerol (~5 mol%) and erythritol (~20 mol%). P.c. (solvent *D*) of the hydrolysate showed a trace of glycerol but no erythritol. The presence of some free glycerol in SD*A* was indicated by p.c. and by g.l.c. of an acetylated sample, which also confirmed the absence of erythritol.

After storage of SD*A* in the acid form at room temperature for ~6 weeks, t.l.c. showed the appearance of some components having mobilities (solvent *E*) close to that of raffinose, although most of the sample did not migrate. Fractionation with 2-propanol (found to be more effective than EtOH or 1:1 MeOH–Me_2CO) yielded SD*A*-1 (235 mg) and a soluble fraction (40 mg). The latter fraction had $[\alpha]_D$ +10°, $\overline{M}_w$ ~620 (see Scheme 1), and was resolved into two components having molecular



Scheme 1.
Stages of depolymerisation in Smith degradation of partially hydrolysed *G. robusta* gum; the products are aligned according to the scale of molecular weights shown.

weights ~670 (88% by weight) and 280 (by s.e.c., Bio-Gel P-10), with no material of higher molecular weight being detectable by t.l.c. or s.e.c. This fraction was similar (by p.c. and g.l.c. of a hydrolysate) in composition to SD*A*-1, which had $[\alpha]_D$ +68°, $\bar{M}_w$ 4400 (single, sharp peak in s.e.c.; t.l.c. showed no low-molecular-weight components). SD*A*-1, which was stored in neutral form at 0° to prevent further spontaneous disaggregation, was used in all further experiments.

P.c. of a hydrolysate of SD*A*-1 indicated a composition identical to that of SD*A*. The presence of glycolaldehyde was suggested by the observation of a streak shortly after a paper run in solvent *D* had been sprayed with ammoniacal $AgNO_3$. P.c. (solvent *C*, development for 4 h only) revealed components (R_F ~0.7 and 0.8) more mobile than D-glucurono-6,3-lactone (R_F ~0.5). One portion of the hydrolysate, after reduction ($NaBH_4$) and acetylation, contained (g.l.c.) derivatives of Gal, Man, erythritol, and glycerol in proportions similar to those in the reduced and acetylated products of hydrolysis of SD*A*, but no erythritol tetra-acetate was detected on g.l.c. of a second portion, acetylated after conversion of the sugars into aldononitriles[12], so that treatment with $NaBH_4$ was omitted. A third portion was trimethylsilylated[26] (in dimethyl sulfoxide[27]) and, when examined by g.l.c. (column *4*) using the following programme, 120° for 15 min, 120 → 200° at 5°/min, 200° for 15 min, gave a peak having the retention time expected for trimethylsilylated erythronolactone[27,28] in addition to those due to trimethylsilylated glycerol, Man, and Gal.

T.l.c. (2-butanone–H_2O azeotrope, acidified with HOAc) of SD*A*-1, with

detection[1] of lactones by hydroxamate-Fe^{3+} reagent[29], revealed a lactone which remained at the origin (*cf.* R_F ~0.6 for D-glucurono-6,3-lactone). The presence of glycolaldehyde was confirmed by assay[17], before and after hydrolysis (M trifluoroacetic acid, 100°, 2 h, in sealed tubes), which released glycolaldehyde corresponding to 10.9% of SD*A*-1 (~8 mol.mol^{-1}); only 5.5% was detected when the hydrolysis was carried out as in the analytical procedure (0.34M trichloroacetic acid, 100°, 30 min). This demonstrated the occurrence of covalently bound glycolaldehyde.

A sample (52 mg) of SD*A*-1 was methylated and one portion of the product (42 mg), $[\alpha]_D$ +42°, was hydrolysed, reduced ($NaBH_4$), and acetylated, the products being analysed by g.l.c. and g.l.c.-m.s. A second portion was submitted to carboxylate-reduction[23] prior to hydrolysis and similar analysis. The third portion was treated with 1,5-diazabicyclo[5.4.0]undec-5-ene in benzene, in the presence of acetic anhydride to prevent, by simultaneous acetylation at O-1, further degradation of the reducing sugars exposed by base-catalysed β-elimination[30,31]. De-acetylation (sodium methoxide) and simultaneous labelling of these reducing sugars by reduction with $NaBD_4$ was followed by mild hydrolysis (aqueous 10% HOAc, 100°, 1 h), to remove the unsaturated acidic residues, and deuteriomethylation of all newly exposed hydroxyl groups before hydrolysis, reduction ($NaBH_4$), acetylation, and g.l.c.-m.s.

*Further degradation of SD*A*-1.* — (*a*) A sample (25 mg) of SD*A*-1 was treated with M trifluoroacetic acid (5 mL) at room temperature for 3 weeks. Aliquots (1 mL and 50 μL, respectively) were removed at intervals of 1 week for s.e.c. (Bio-Gel P-10) and assay for glycolaldehyde. Slow breakdown to small fragments ($\overline{M}_w$ ~600) and release of virtually all bound glycolaldehyde (10.7% of SD*A*-1) were observed, with negligible liberation of Gal and Man.

(*b*) A solution of SD*A*-1 (100 mg) in M trifluoroacetic acid (10 mL) was stored at room temperature for 3 weeks. All of the glycolaldehyde (10.9%) had then been released and s.e.c. (Bio-Gel P-2) revealed a mixture of small carbohydrate-containing fragments, $\overline{M}_w$ ~600. After removal of the acid (by freeze-drying), the residue (~80 mg) was treated with $NaBD_4$ (200 mg) in dry methanol (5 mL) for 65 h; the solution was then acidified (HOAc), borate was removed by co-distillation with MeOH, and cations were removed with Amberlite IR-120 (H^+) resin. Freeze-drying then yielded SD*A*-2 (36 mg), which had negligible i.r. absorbance for carboxylic acid or lactone (1700–1780 cm^{-1}). P.c. (solvent *D*) of a hydrolysate revealed erythritol in appreciable proportion. The ^{1}H-n.m.r. spectrum (Bruker WH-90 spectrometer; internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate) of a deuterium-exchanged sample of SD*A*-2 in D_2O at 80° contained prominent signals at δ 5.05 [d, *J* 1.8 Hz, H-1 of α-D-Man*p*-(1→2)-][32] and 5.23 (characteristic[33] of 2,3-linked α-D-Man*p* contiguous to polyol at the reducing end), and other signals in the ranges 5.17–5.36 and 4.7–4.9 which account for Man*p* and Gal*p* residues in other linkage modes (see below).

*Fractionation of SD*A*-2.* — Preparative s.e.c. of SD*A*-2 (25 mg) on Trisacryl GFO5 gave four components (I–IV) having mol. wts of ~1250, 700, 400, and 250,

in the mass ratios ~4:6:3:1 ($\overline{M}_w$ ~750). The fractions were isolated by freeze-drying and investigated by t.l.c., determination of $[\alpha]_D$, uronic acid, and glycolaldehyde, g.l.c.–m.s. of the reduced ($NaBH_4$), acetylated products of hydrolysis, and methylation analysis (following treatment of methylated I with $LiAlD_4$). Methylated IV was examined by e.i.-m.s. before g.l.c.–m.s. of the reduced and acetylated products of hydrolysis.

Degradation of carboxyl-reduced SDA-1. — Treatment of SD*A*-1 (20 mg) with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide[34] at pH 4.75, followed by reduction with 3M $NaBH_4$ at pH 7.0, yielded a product that contained (g.l.c.–m.s. of the acetylated products of hydrolysis) erythritol in high proportion and a trace of Glc in addition to Man and Gal. Treatment of the bulk (~15 mg) of this product with M trifluoroacetic acid at room temperature for 65 h caused breakdown into

TABLE I

ANALYTICAL DATA FOR *Grevillea robusta* GUM POLYSACCHARIDE AND PARTIALLY HYDROLYSED POLYSACCHARIDE, *A*

	Whole gum	A
$[\alpha]_D$ (degrees)	−8	+34
$\overline{M}_w$[a] × 10^5	15	2.7
Uronic acid (mol %)	16	24
Neutral sugars (mol %)[b]		
Rha	2	1
Ara	44	13
Xyl	2	3
Man	6	14
Gal	30	45
Modes of linkage[c] *(mol %)*[d]		
Ara*f*→	10	7
Ara*p*→	11	6
Xyl*p*→	2	3
→5)-Ara*f* / →2)-Ara*p*	10	—
Gal*p*→	4	10
→2)-Man*p*	2	5
→3)-Gal*p*	5	7
→6)-Gal*p*	17	20
→2,3)-Man*p*	2	6
→3,6)-Gal*p*	4	3
Other	[e]	[f]

[a]From s.e.c. (Sepharose 4B; dextran calibration). [b]By g.l.c. of alditol acetates (column *1*). [c]By g.l.c. of partially methylated alditol acetates (column *2*); identities, indicated by retention times relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, verified by mass spectrometry. [d]Adjusted to allow for proportions of uronic acid. [e]Traces (<3%) of Rha*p*→; →2)- and →3)-Ara*f*; →4)-Gal*p*; →3,4)-, →4,6)-, and →2,6)-Gal*p*; →2,6)-Man*p*; →3,4,6)- and →2,4,6)-Gal*p*; →2,3,6)-Man*p*. [f]Traces of Rha*p*→; →3,4)-, →4,6)-, and →2,6)-Gal*p*; →2,6)-Man*p*; →3,4,6)-Gal*p*.

SD*A*-2', which contained components having mol. wts. (from s.e.c. on Bio-Gel P-2) of ~1300 (trace), 700, 400, and 270 (mass ratios ~6:3:1); $\overline{M}_w$ ~600 (see Scheme 1). Further treatment with cold acid (for 80 h) caused no change in this molecular-weight distribution.

RESULTS AND DISCUSSION

The analytical data for the *Grevillea robusta* gum polysaccharide in Table I are similar, in general, to those reported previously[5] and also those of another specimen of *G. robusta* gum[6]; the higher $\overline{M}_w$ (88×10^5) recorded for the latter, which may be related to its production by a tree growing in artificial conditions under glass[6], is the only significant difference between these two specimens. As expected, partial acid hydrolysis removed most of the Ara residues, and some Gal, free or in small oligosaccharides, was also released, but no Man or uronic acid, which were present in enhanced proportions in polysaccharide *A* (Table I). The Man*p* residues occurred mainly as 2-linked chain units or 2,3-linked branch points, and the uronic acid residues as end groups or 4-linked chain units (in the molar ratio 7:8 as indicated by g.l.c. of the derived methyl ester methyl glycosides[5]).

Owing to the removal of protecting, peripheral residues, the extent of depolymerisation on Smith degradation of *A* was considerably greater than that for the parent gum[5], which caused $\overline{M}_w$ to decrease to ~470,000 (indicating minimal internal fission in addition to the removal of peripheral groups). Three sequential Smith-degradations of the whole gum were required to remove the Ara residues, at which stage $\overline{M}_w$ had fallen to ~2000. Nevertheless, the accumulation of Man*p* residues in the degraded polysaccharide was evident even after the first Smith-degradation[5].

Under standard conditions[25], Smith degradation of *A* caused $\overline{M}_w$ to fall to 4400 and the proportion of Man*p* residues to increase to ~40 mol%. As noted earlier[5], some uronic acid residues survived, but the production of a high proportion of erythritol on borohydride reduction of the products of hydrolysis of SD*A* and SD*A*-1 indicated that most of the acidic residues were present as erythronic acid units, formed from periodate cleavage of 4-linked Glc*p*A[35,36]. The absence of free erythritol and the detection, in the hydrolysate of SD*A*-1, of a component having the chromatographic mobility (p.c.; g.l.c. of the trimethylsilyl derivative) of erythronolactone confirmed that erythritol present among the reduced products of hydrolysis arose from this source. That these erythronic acid units were acetal-linked through glycolaldehyde to the next sugar residue in the chain in SD*A*-1 was corroborated by the detection of glycolaldehyde bound within the molecule. Such acetals have been found amongst the products of Smith degradation of *Prunus* gums[1], and their resistance to hydrolysis by cold acid has been ascribed[1] to lactonisation. The presence of lactones in SD*A*-1, as in Smith-degraded *Prunus* armeniaca gum[1], was suggested by the positive reaction in the hydroxamate–Fe^{3+} test[29] of the material which remained at the origin in t.l.c. under conditions that

TABLE II

METHYLATION ANALYSES OF SD*A*-1 AND PRODUCTS OF CARBOXYLATE-REDUCTION AND BASE DEGRADATION

Sugar linkage[a]	*Molar proportions (%)*[b]		
	Methylated SDA-1 (MSDA-1)	*Carboxylate-reduced MSDA-1*[c]	*Base-degraded MSDA-1*[d]
Man*p* →	7	10	12
Gal*p* →	26	20	27
→2)-Man*p*	7	6	trace
→3)-Man*p*	5	6	10
→3)-Gal*p*	3	3	3
→4)-Gal*p*	2	3	3
Glc*p*[e]→	—	3	—
→6)-Gal*p*	2	3	2
→2,3)-Man*p*	19	20	3
→4)-Glc*p*[f]	—	3	—
→4,6)-Gal*p*	3	3	3

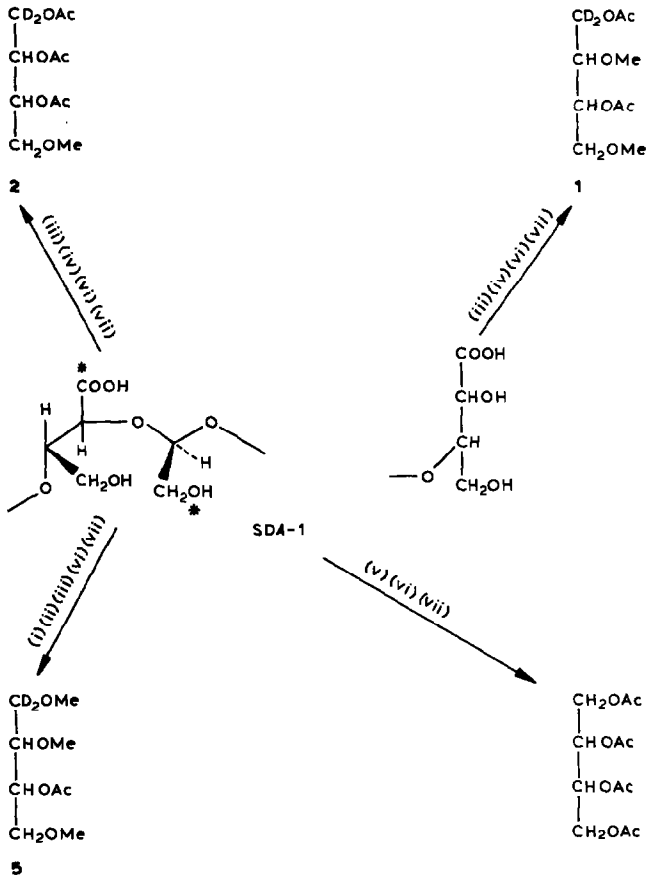
[a]By g.l.c. of partially methylated alditol acetates (column *3*); identities, from retention times relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, confirmed by m.s. [b]Adjusted to allow for proportions of Glc*p*A (6%) and erythronic acid (~20%). [c]Also showed peaks (relative retention times, 0.44 and 0.54) due to **1** and **2**. [d]Deuterated mannitol derivatives **3** (8 mol%) and **4** (3%) also present; relative retention times, 0.56 and 0.62. [e]From terminal Glc*p*A; deuterated at C-6. [f]From →4)-linked Glc*p*A; deuterated at C-6.

cause lactones of low molecular weight to migrate.

Methylation analysis of SD*A*-1, before and after carboxylate-reduction of the methylated derivative (Table II), showed terminal Gal*p* groups and 2,3-linked Man*p* branch-points to be the major constituents, with Man*p* residues as end groups and as 2- and 3-linked units also present in appreciable proportions. The few surviving uronic acid residues occurred both as terminal groups and as 4-linked units. Carboxylate-reduction of methylated SD*A*-1 also gave two components having g.l.c. retention times of di- and mono-methyl ethers of erythritol, the latter preponderating; the corresponding mass spectra indicated structures **1** and **2**, respectively (see Scheme 2). Characteristic ions are: **1**, *m/z* 43 (100%), 45 (20), 59 (10), 89 (30), 101 (5), 119 (15), 131 (15), 161 (trace), 191 (2); **2**, *m/z* 43 (100%), 45 (35), 57 (5), 75 (5), 87 (30), 102 (35), 117 (75), 129 (25), 144 (20), 147 (10), 162 (5), 177 (5), 219 (5).

The labelling consequent on reduction with $LiAlD_4$ showed both **1** and **2** to originate from the erythronic acid units in SD*A*-1. The production of **1** from a terminal unit and of **2** from acetal-linked erythronic acid within the chain is illustrated in Scheme 2. It should be noted that the existence of terminal erythronic acid (albeit in small proportion) in SD*A*-1 reflects the occurrence of some acid hydrolysis of the corresponding glycolic acetal.

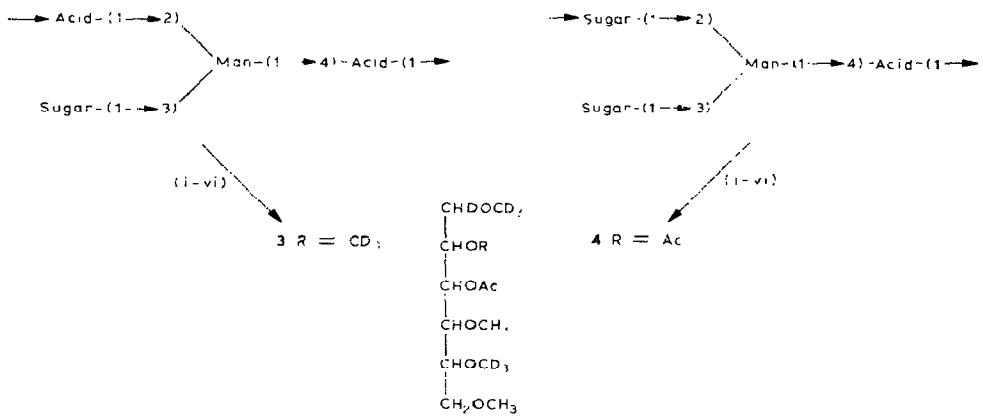
The procedure used in the base-catalysed degradation of esterified and methylated SD*A*-1 included a minor modification of Aspinall's approach[30,31] to isolating



Scheme 2.
Production of various derivatives of erythritol from combined erythronic acid (lactonisation also possible at *) in SD*A*-1. Chemical changes brought about by (i) acetal hydrolysis, (ii) borodeuteride reduction, (iii) methylation, (iv) reduction with lithium aluminium deuteride, (v) carboxyl-reduction, (vi) hydrolysis, and (vii) acetylation.

fragments both exterior and interior to the points of cleavage. Sugars detached by β-elimination from positions exterior to the acidic residues were labelled with deuterium at C-1 before the removal, by mild acid hydrolysis, of the unsaturated acidic moieties remaining attached to interior sugar units. In this sequence, the alditol formed by borodeuteride reduction was subsequently further labelled by deuteriomethylation at positions 1 and 5, and, if the parent sugar was located interior to acid, the position to which the acid had been joined.

The results of g.l.c.-m.s. analysis of the derivatised products (Table II) show the juxtaposition of Man*p* (M) with respect to contiguous acid (A) or sugar (S) residues to vary as follows. (*a*) Almost complete disappearance of 2-linked Man*p* and a ~5% increase in the molar proportion of terminal Man*p*, with the introduc-



Scheme 3.
Production of **3** and **4** from SD*A*-1 after (i) base-catalysed degradation of methylated derivative, (ii) borodeuteride reduction, (iii) hydrolysis of unsaturated acid moieties, (iv) deuteriomethylation, (v) hydrolysis, (vi) borohydride treatment and acetylation. Definitive ions: **3**, *m/z* 43 (80%), 45 (40), 49 (18), 61 (12), 64 (40), 92 (20), 96 (100%), 101 (30), 104 (80), 109 (10), 117 (40), 135 (18), 136 (20), 164 (20), 177 (25), 209 (5), 212 (60), 259 (15); also 93 (15), significance of which is discussed in the text; **4**, 43 (100%), 45 (20), 49 (60), 81 (30), 82 (20), 84 (50), 85 (55), 92 (12), 95 (30), 96 (20), 97 (55), 99 (30), 101 (15), 104 (60), 109 (20), 111 (35), 113 (20), 125 (25), 127 (15), 136 (12), 139 (15), 269 (15).

tion of CD_3O groups at position 2, reflects the presence of A→2M→S. (*b*) The molar proportion of 2,3-linked Man*p* fell from 19 to ~3%, whilst (*1*) the proportion of 3-linked Man*p* rose from 5 to 10%, with the introduction of CD_3O groups at position 2, corresponding to A→2M→S, (*2*) ~8 mol% of component **3** (a substitu-
3
↑
ted mannitol, reflecting the presence of A→2M→A; see Scheme 3) appeared, and
3
↑
(*3*) ~3 mol% of component **4** appeared (Scheme 3), showing the occurrence of S→2M→A. In (*1*) - (*3*), the Man*p* residue carried a sugar at position 3 and the loss
3
↑
of 2,3-linked Man*p* was entirely accounted for. The structural implications of these different distributions are discussed below in the context of the partial structures proposed for fragments of SD*A*-1 liberated on further breakdown by cold acid.

Smith degradation of polysaccharides containing 4-linked Glc*p*A units (*e.g.*, gum ghatti[37]) is complicated by the very low rate of hydrolysis with cold acid of the acetals produced from these units. This phenomenon was observed in the Smith degradation of partially hydrolysed *Prunus armeniaca* gum[1]; the product, $\overline{M}_w$ ~6000, obtained after degradation under standard conditions, was disaggregated slowly on long storage in the acid form or prolonged treatment, in solution, with acid, eventually reaching a second limit of depolymerisation, $\overline{M}_w$ ~1000 (two major components had molecular weights ~1100 and 500). The appearance of components of lower molecular weight in the 2-propanol-soluble fraction isolated after the

acid form of SD*A* had been stored at room temperature for ~6 weeks indicated that the product having $\overline{M}_w$ 4400 represented only the first limit of Smith degradation.

Treatment of SD*A*-1 with cold acid for 3 weeks cleaved the erythronic acid–glycolaldehyde acetals, with release of glycolaldehyde and fragmentation into small molecules. When this second-limit Smith-degradation product was treated with $NaBD_4$, there was evidence (from hydrolysis and p.c.) for the formation of erythritol from erythronolactone at the reducing ends of the molecules. Fractionation of SD*A*-2 by s.e.c. yielded fractions I–IV (Tables III and IV), each of which was found (by g.l.c.–m.s. of the reduced, acetylated hydrolysate) to contain erythritol deuterated at a primary alcohol site, with a little erythritol produced by subsequent reduction ($NaBH_4$) of unchanged erythronolactone. The intensities of the deuterated ions, *m/z* 75, 117, and 219, relative to those of the ions *m/z* 73, 115, and 217 in the mass spectrum of erythritol tetra-acetate indicated ~80% deuteration at one primary alcohol site. No deuteration was evident from the mass spectra of the hexitol hexa-acetates in the reduced and acetylated products of hydrolysis, indicating that erythronolactone constituted the terminal group in each of the components of the second-limit Smith-degradation product.

That the acidic acetals in SD*A*-1 are responsible for its very low rate of hydrolysis in cold acid is further indicated by the accelerated breakdown observed after carboxyl reduction[34]. Treatment of carboxyl-reduced SD*A*-1 with acid for <3 days effected depolymerisation comparable with that reached only after 3 weeks for SD*A*-1 (see Scheme 1). This procedure offers a means of expediting Smith degradation of polysaccharides containing 4-linked Glc*p*A residues.

Methylation analysis of II–IV and carboxylate-reduced methylated I revealed

TABLE III

ANALYTICAL DATA FOR FRACTIONS I–IV FROM SD*A*-2

	I	*II*	*III*	*IV*
Yield (mg)	3.6	5.7	3.7	1.2
R_F (t.l.c)[a]	0.24	0.24–0.32[b]	0.41	0.50
$[\alpha]_D$ (degrees)	+28	+24	+24	+23
$\overline{M}_w$[c]	1250	700	400	250
Residual glycolaldehyde (mol.mol^{-1})	0.12	—	—	—
Uronic acid (mol%)	12	4	—	—
Other constituents (mol%)[d]				
Ery[e]	16	24	35	38
Man	38	36	33	44
Gal	34	36	32	18[f]

[a]Silica gel, solvent *E*; R_F values for raffinose, cellobiose, and glucose were 0.37, 0.45, and 0.52, respectively. [b]Streak, indicating a mixture. [c]Estimated from s.e.c. on Trisacryl GF05. [d]By g.l.c. (column *3*) of hydrolysate after reduction ($NaBH_4$) and acetylation; identification from standards confirmed by m.s. [e]Deuterated at a primary alcohol site in ~80% of molecules. [f]Due to presence of some III mixed with IV.

TABLE IV

METHYLATION ANALYSES OF II–IV AND CARBOXYLATE-REDUCED METHYLATED I

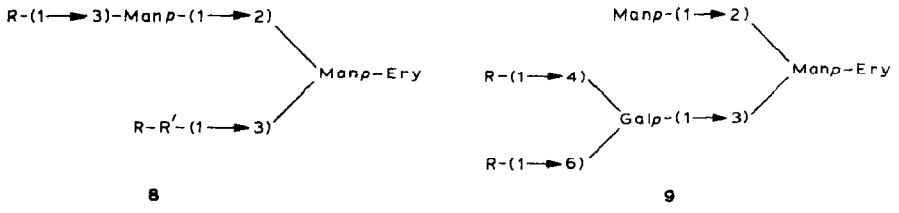
Sugar linkage	*RMI*[a]	*II*	*III*	*IV*
Man*p*→	+	+	–	+++
Gal*p*→	++	++	+++	trace[c]
→3)-Man*p*	+	+	+++	trace[c]
→3)-Gal*p*	–	trace	–	–
→4)-Gal*p*	–	trace	–	–
→6)-Gal*p*	–	trace	–	–
→2,3)-Man*p*	+	++	–	–
→4)-Glc*p*[b]	+	–	–	–
→4,6)-Gal*p*	+	trace	–	–

[a]Carboxylate-reduced, methylated I. [b]From →4)-linked Glc*p*A; deuterated at C-6. [c]Due to presence of some III.

sugar residues linked as indicated in Table IV. The reduced, acetylated products of hydrolysis of methylated I–IV also contained, in significant proportion, another component (g.l.c. retention time ~0.4 relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol) that appeared from its mass spectrum to have structure **5**, derived from the terminal erythronic acid groups as shown in Scheme 2. Characteristic ions are *m/z* 43 (100%), 45 (15), 47 (7), 61 (10), 71 (55), 91 (12), 99 (40), 101 (70), 117 (70), 129 (35), 131 (5), 161 (55), 163 (10).

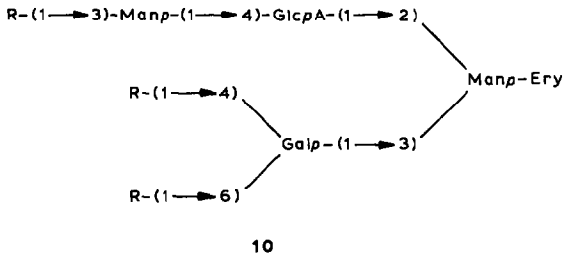
E.i.-m.s. of methylated IV produced ions (*m/z* 45, 58, 91, 149, and 219) indicative of a methylated hexopyranosyl-erythritol. From the data shown in Tables III and IV and the evidence from ^{1}H-n.m.r. spectroscopy of SD*A*-2, which corroborates that from the specific optical rotations of I–IV, it is concluded that the hexopyranose residue is α-D-Man*p*. The analytical results for III show the attachment of a single β-D-Gal*p* residue (inferred from the specific optical rotation) at position 3 of α-D-Man*p* linked to erythritol as in IV; IV and III are thus α-D-Man*p*-Ery (**6**) and β-D-Gal*p*-(1→3)-α-D-Man*p*-Ery (**7**), respectively.

T.l.c. (Table II) and the complex mixture of methylated sugar residues identified on methylation analysis (Table IV) indicate that fraction II is a mixture. Terminal Gal*p* and 2,3-linked Man*p* are major components, with terminal and



3-linked Man*p* also present in significant proportions, but the presence of only traces of methyl ethers corresponding to Gal*p* residues as chain units, 3-, 4-, or 6-linked, or 4,6-linked branch-points suggests that such residues occur in some, but not all, of the components of II. Examples of possible structures present in II are **8** and **9**. The distribution of Man*p* and Gal*p* amongst R and R′ is such that the overall ratio in II is 1:1. The specific optical rotation of II indicates that the Gal is β-D-Gal*p*, but D-Man*p* not linked to Ery is α or β. It may be inferred from the n.m.r. evidence that the 2,3-linked Man*p* residue linked to Ery is α-D-Man*p*[33].

Although the intact Glc*p*A residues in SD*A*-1 occur not only as 4-linked chain units but also as terminal groups (Table II), methylation analysis of carboxylate-reduced methylated I afforded no evidence of the latter. A possible formulation for I is **10**.



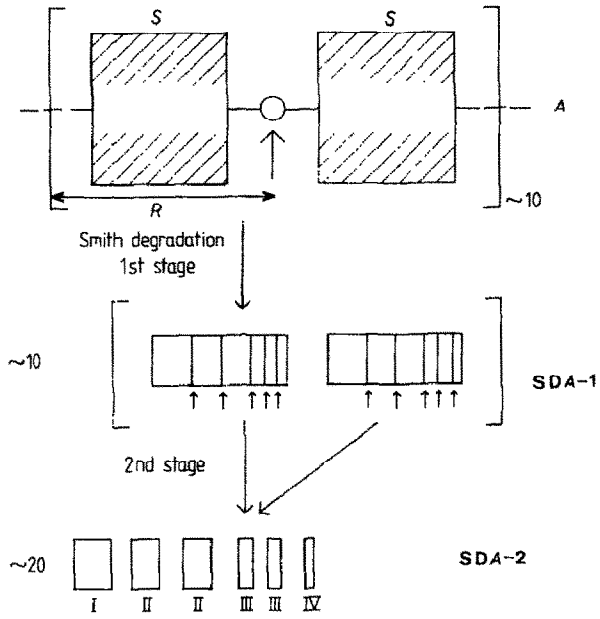
10

R = Man*p* or Gal*p* (Man:Gal = 1:1)

A smaller fragment, without the 3-linked Man*p* exterior to Glc*p*A, may also occur among the components of SD*A*-2; in s.e.c., this would be eluted in Fraction II, which contained a small proportion of uronic acid (see Table III). Therefore, the terminal Glc*p*A groups may be present in II attached to 2,3-linked Man*p*; no evidence for their attachment to Gal*p* was afforded by the labelled products of base-catalysed degradation of methylated SD*A*-1.

The molar proportion of erythritol found in the reduced products of hydrolysis of SD*A*-1 indicated the presence of ~6 erythronic acid units per molecule, with one at the reducing end and ~5 involved in acetal linkages, through glycolaldehyde, in the main chain. The proportions of fragments I–IV, obtained after prolonged treatment of SD*A*-1 with acid, suggest that the corresponding sub-units in the molecular structure occurred in the molar ratios 1:2:2:1, as illustrated in Scheme 4, which also depicts the production of SD*A*-1 from *A*, after Smith degradation to the first limit.

From the proportion of periodate-vulnerable sugars in *A* (~64%, according to methylation analysis; Table I), removed during Smith degradation to the first limit, SD*A*-1 (molecular weight ~4400) corresponds to a repeating sub-unit *S* (Scheme 4), having a molecular weight of ~12,000. The number of acidic residues present in SD*A*-1 (~7, including unchanged 4-linked Glc*p*A) represents ~66% of the total 4-linked Glc*p*A per hypothetical "repeating unit" *R* (Scheme 4) in *A*,



Scheme 4.
Diagrammatic representation of the sizes of the products at various stages of depolymerisation. For *A*, the rectangle represents sub-unit *S*, mol. wt. ~12,000, and hatched areas denote the periodate-vulnerable sugars in the side chains. The hypothetical repeating unit *R* includes periodate-vulnerable sugars, –O– in the main chain. For SD*A*-1, each rectangle represents an assembly of residues having a mol. wt. of ~4400, the strips correspond to fragments I–IV in SD*A*-2, linked through acetals of glycolaldehyde, and ↑ indicates the points of cleavage on prolonged treatment with cold acid.

leaving up to four 4-linked Glc*p*A residues in regions outside the uniform, periodate-resistant blocks that yield SD*A*-1. The percentage of 2-linked Man*p*, lost from *A* during Smith degradation to SD*A*-1, is concomitant with this proportion of 4-linked Glc*p*A. It is possible, therefore, that up to four contiguous →4)-Glc*p*A-(1→2)-Man*p*-(1→ units may alternate with the sub-unit *S*, of which ~20 occur per molecule of *A*.

The products of base-catalysed degradation of the esterified units in methylated SD*A*-1 demonstrate that each of the portions of SD*A*-1, giving rise to I–IV on cleavage of the acidic acetal systems when Smith degradation proceeds to the second limit (Scheme IV), is linked to the next through position 2 of Man*p*. The presence of Man*p* residues located as in **6–10** is further corroborated as follows. (*a*) Both **10** and (if located in the interior) **7** contain 2,3-linked Man*p* in the situation A→2M→A,
3
↑
the expected product of base-catalysed degradation, **3**, being present among the observed products in a proportion comparable with that predicted (~11%) from a consideration of the composition of SD*A*-1 as indicated in Scheme 4. (*b*) If **10** or **7** is at the external end of the molecule, the 3-linked Man*p* should yield a mannitol

derivative differing from **3** only in carrying $-OCH_3$, not $-OCD_3$, at position 2. This inference is substantiated by the ion *m/z* 93 (15%) in the mass spectrum, which distinguishes this derivative from **3** for which the corresponding ion at *m/z* 96 is the base peak. A small proportion of 3-linked Man*p* in a sequence S→3M→A is thereby indicated. (*c*) Both **8** and **9** contain 2,3-linked Man*p* located as S→2M→A; the

3
↑

expected product, **4**, was observed, although its molar proportion (~3%) was low compared with the predicted 7%. The presence of some (~3%) unchanged 2,3-linked Man*p* may reflect less than quantitative reduction by borodeuteride of these sugar residues after liberation from contiguous acid units. (*d*) Deuteriomethylation at position 2 of previously 2- and 2,3-linked Man*p*, without borodeuteride reduction, corroborates the postulation of Man*p* residues exterior to the 2,3-linked Man*p* in **8**

and **9**; these Man*p* residues would be located A→2M→S and A→2M→S, if **8** and

3
↑

9, respectively, were interior to acid units. The observed molar proportion (~5%) of each of the corresponding deuteriomethylated Man*p* derivatives agrees with that (~4%) predicted for SD*A*-1. (*e*) The Man*p* residue in **6** should yield a product with the g.l.c. retention time of hexa-*O*-methylmannitol, eluting marginally ahead of tri-*O*-acetylglycerol (relative retention times, 0.43 and 0.45, respectively). This will differ from **3** in carrying $-OCH_3$ at position 3 and, if **6** is at the external end of SD*A*-1, also at position 2. Amongst the products of similar base-catalysed degradation and labelling at a corresponding stage in the Smith degradation of *Prunus armeniaca* gum[1], m.s. evidence was obtained for the latter. No such mannitol derivatives were detected here, although the proportion of 2-linked Man*p* situated A→2M→S [see (*d*)] does not account entirely for the total lost on base-catalysed degradation of SD*A*-1 and, therefore, it is possible that some may have been located A→2M→A, as required for the production of fragment IV in SD*A*-2.

It is concluded that the structure of SD*A*-1 in which fragments **6** - **10** in the molar ratios ~1:2:1:1:1 are acetal-linked, each to position 2 of Man*p* in the succeeding unit, fits the experimental data for both SD*A*-1 and SD*A*-2. With 4-linked Glc*p*A in the positions occupied by the acidic acetals in SD*A*-1, this model may be regarded as representative of the repeating blocks in the core of *A*, and hence of the *Grevillea robusta* gum polysaccharide. This resembles *Prunus armeniaca* gum[1] in containing Man*p* and Glc*p*A residues, most of the former carrying short side-chains at position 3. The proportions in SD*A*-1 of **6**, **7**, and **10**, each of which contains Man*p* residues in the A→2M→A or A→2M→A situation (with two pos-

3
↑

sible in **10**), indicate that the corresponding block in the polysaccharide core could contain up to five contiguous →4)-Glc*p*A-(1→2)-Man*p*-(→ units. With four that may possibly occur, as noted earlier, in the regions that do not survive Smith degradation to the first limit, the core of the polysaccharide could contain as many

as nine contiguous aldobiouronic acid units per repeating unit *R*. However, the presence of structures of the type represented by **8** and **9** demonstrates periodic interruption of the glucuronomannoglycan sequence by adjacent Man*p* residues. Investigation of polysaccharide *A*, by other methods of selective cleavage of glucopyranosiduronic acid linkages[38], and partial acid hydrolyses, may afford further evidence for this core structure.

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