Carbohydrate Research, 158 (1986) 157–171
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THE GLYCOPROTEINS OF *Acacia erioloba* EXUDATES*

DAVID W. GAMMON, ALISTAIR M. STEPHEN, AND SHIRLEY C. CHURMS
Department of Organic Chemistry, University of Cape Town, Rondebosch 7700 (South Africa)
(Received December 7th, 1985; accepted for publication, August 2nd, 1986)

ABSTRACT

The gum exudate from *Acacia erioloba* (syn. *giraffae*) contains protein (53–56%) and carbohydrate as a mixture of glycoproteins that are relatively resistant to proteases and not readily separable. The protein components have high contents of hydroxyproline and serine, and the carbohydrate is composed mainly of L-arabinose, D-galactose, and 4-*O*-methyl-D-glucuronic acid residues. Methylation, partial acid hydrolysis, and alkaline hydrolysis studies revealed units of the aldobiouronic acids α-4MeGlc*p*A-(1→4)-Gal and β-4MeGlc*p*A-(1→6)-Gal, and that the carbohydrate, mainly in the form of short, linear oligosaccharides, is attached to Hyp residues in the peptide chains. This gum exudate differs markedly from others derived from the same taxonomic series (Bentham's Series 4) of *Acacia*.

INTRODUCTION

Plant biopolymers containing L-arabinose and D-galactose occur in three broad structural categories[1,2]: polysaccharides, exemplified by numerous *Acacia* exudates; arabinogalactan–proteins (AGP's) such as those excreted into the medium by cultured cells[3], which have a low protein content (<10%) and are thus classified as proteoglycans; and glycoproteins containing higher proportions of protein covalently associated with oligosaccharides, such as the lectin of potato tubers *(Solanum tuberosum)*[4].

The heterogeneity of gum arabic from *A. senegal* (Bentham's Series 5: Vulgares) is due to association of some of the carbohydrate with protein[5], and the attachment of both polysaccharide and oligosaccharides through *O*-glycosylic linkages to protein-bound hydroxyproline (Hyp) has been demonstrated[6]. The contents of protein and amino acid analyses for several *Acacia* gums have been reported[7–9] and a feature of these, and of *A. robusta* gum[10], is the high proportion of hydroxyproline, serine, and aspartic acid. Of particular interest are several gums from the sub-series Juliflorae of Series 1 (Phyllodineae), which have high contents of protein (40–53% by weight), negative optical rotations, and high proportions of

*Presented, in part, at the International Workshop on Plant Polysaccharides, Nantes, France, July, 1984, and the 8th International Symposium on Glycoconjugates, Houston, U.S.A., September, 1985.

0008-6215/86/$ 03.50

4MeGlcA (9–33% of total carbohydrate)[11], features found in the gum of *Acacia erioloba* described here and also (incidentally) in the gum of *Azadirachta indica*[12] (Meliaceae: Sapindales).

Our studies have focussed on *Acacia* gums from Series 4 (Gummiferae), having contents of protein varying from <2% *(A. karroo)*[13], through 12% *(A. tortilis)*[14] and 18% *(A. robusta)*[10], to 55% in *A. erioloba*, reported here. The first three have structures typical of *Acacia* polysaccharide exudates, although a small proportion of arabinosides joined directly to peptidyl Hyp was detected in the gum of *A. tortilis*. In both *A. robusta* and *A. tortilis* gums, there is evidence for covalent association of polysaccharide and protein.

In previous investigations[15] of gum samples from *A. erioloba* (syn. *giraffae*) having low contents of protein, the polysaccharide had a positive $[\alpha]_D$ value, yielded, on hydrolysis, all four of the aldobiouronic acids α-D-Glc*p*A-(1→4)-D-Gal, β-D-Glc*p*A-(1→6)-D-Gal, and their analogues containing 4MeGlc*p*A, and contained a preponderance of terminal, non-reducing Ara*f* and branched (1→3),(1→6)-linked Gal*p* residues. The marked contrast of several of these features to those of two recently collected samples of this gum prompted a further, detailed structural examination.

EXPERIMENTAL

General methods. — P.c. was performed on Whatman No. 1 paper with *A*, ethyl acetate–pyridine–water (8:2:1); and *B*, 1-butanol–acetic acid–water (2:1:1). G.l.c. was performed on *1*, a column (2 m × 3 mm i.d.) of 3% of OV-225 on Chromosorb W-HP (80–100 mesh); and *2*, a quartz capillary column (30 m × 0.32 mm i.d.) with OV-225 (0.25-μm film thickness) as the bonded phase (Durabond DB-225; J. and W. Scientific).

Determination of the proportions of neutral sugars in hydrolysates, amino acid analyses, methylations, base degradations of methylated products, and carboxyl-reductions in methylated products were as described previously[10,14,16]. The proportions of neutral and acidic sugars were found after methanolysis[17]. 90-MHz ^{1}H-N.m.r. spectra (internal acetone, δ 2.20) were recorded for deuterium-exchanged specimens, using a Bruker WH-90 spectrometer.

Origin and isolation of gum samples. — One sample, from a torn branch of a small tree growing near Kimberley, South Africa, was collected by Mr. A. Gubb of the Kimberley Museum, who confirmed the species of origin as *Acacia erioloba* E. Mey. A portion (1.21 g) was suspended in cold water and stirred for 24 h. The insoluble material was removed, and the filtrate was dialysed for 48 h against running tap-water and then freeze-dried to give *A* (0.91 g). Ammonium sulphate fractionations were performed on a sample obtained by freeze-drying the water-soluble part (*A'*, 1.36 g) of another portion of the crude gum (1.99 g). A second sample was collected, as a bulky exudate, by Mr. R. A. Stevens in Ongwediva, SWA/Namibia, the species of origin being authenticated as *A. erioloba* E. Mey.,

from specimens of leaves, pods, and bark, and from photographs, by the Director of the Botanical Research Institute, Pretoria. Portions of the clear, yellow gum dissolved in cold water to give 0.5% solutions, but yielded clear gels within 1 h. Either the freeze-dried gel or the original gum nodules were used, as gum sample *B*, without further purification.

Analytical results are shown in Table I. The amino acid residues in *A* and *B*, and their proportions (mol % of total amino acids) were: Ala (4.3, 4.4), Gly (4.9, 4.8), Val (6.3, 6.1), Thr (4.9, 4.8), Ser (13.2, 13.0), Leu (5.7, 5.6), Ile (3.3, 3.2), Pro (5.9, 6.6), Hyp (22.4, 22.9), Met (0.2, 0.1), Asp (9.4, 9.5), Phe (4.0, 3.8), Glu (4.6, 4.2), Lys (3.5, 3.6), Tyr (3.9, 2.7), Arg (1.9, 2.4), and His (2.1, 2.4).

Electrophoresis (PAGE). — Samples (1–2 mg.mL^{-1}) were prepared for application to polyacrylamide gels (12.5%) by heating them for 10 min at 100° in a solution containing 10% of sodium dodecyl sulphate (SDS), 10% of 2-mercaptoethanol, 15% of glycerol, and 0.01% of Bromophenol Blue in M Tris-HCl (pH 8.6)[18]. These solutions (20–40 μL) were placed in wells on the gel slabs. After electrophoresis (35 mA for 5–8 h), protein and carbohydrate were detected by silver-staining techniques[19].

Haemagglutination assays. — Portions of fractions *A'*, A60P, and A80P were assayed[20] for haemagglutination by using erythrocytes derived from rabbit and human blood (A, B, and O, all positive). The assay was also performed with trypsin- or neuraminidase-treated erythrocytes, and, with each type of erythrocyte, solutions of concanavalin A and *Vicia fabia* lectins and a blank were assayed as controls.

Fractionation of gum sample A. — When a solution of *A* (400 mg) in water (~10 mL) was eluted with water from a column (20 × 1 cm) of Amberlite IR-120 (H^+) resin, the carbohydrate (detected by phenol–H_2SO_4[21]) emerged early as a cloudy suspension which, after centrifugation, yielded water-insoluble A2 (32 mg, 8%) and soluble A1 (272 mg, 68%). Chromatography of A1 and A2 on hydroxyapatite was performed as described previously[10].

To a solution of a portion (500 mg) of *A'* in water (50 mL) was added $(NH_4)_2SO_4$ to 60% saturation; the solution was kept at 4° overnight, and the precipitate was isolated by centrifugation. An aqueous solution thereof was dialysed and then freeze-dried to give A60P (125 mg, 25%). $(NH_4)_2SO_4$ was added to the supernatant solution to ~80% saturation and the precipitate was isolated in the same way to give A80P (100 mg, 20%). Dialysis, with freeze-drying of the non-dialysable portion, gave A80S (160 mg, 32%).

Portions (~100 mg) of A60P, A80P, and A80S were each eluted from a column (36 × 3.2 cm) of DEAE-cellulose, initially with 0.05M Tris-HCl (pH 8.6) and then with a linear gradient (0→0.5M) of NaCl in this buffer. Fig. 1 typifies the elution behaviour of all three samples.

Partial acid hydrolysis. — A1 (210 mg) was heated in aqueous trifluoroacetic acid (~0.01M, 49 mL, pH 2.0) at 100°, aliquots being removed at intervals for determination of $[\alpha]_D$ and examination of hydrolysis products (p.c., solvent *A*). The $[\alpha]_D$

TABLE I

COMPOSITION OF GUM SAMPLES *A* AND *B* FROM *A. erioloba* AND PRODUCTS OF FRACTIONATION OF *A*

	A	B	*PA*[a]	*A1*[b]	*A2*[b]	*A60P*[c]	*A80P*[c]	*A80S*[c]	*A60P3*[d]	*A80P3*[d]	*A80S3*[d]
Yield (wt % of *A*)	—	—	50	68	8	25	20	32	7	10	15
$[\alpha]_D$ (degrees)	−43	−ve	−51	−42	−44	−39	−45	−49	−43	−40	−45
N (%)	9.0	8.5	7.5	8.8	11.2	10.3	9.2	6.2	9.9	7.8	5.8
Protein (%)[e]	56	53	47	55	70	65	58	39	62	49	36
Constituent residues (mol %)				[f]	[f]						
Ara	36	31	45	36	48	n.d.[g]	n.d.	n.d.	51	44	41
Man	7	6	—	5	14	n.d.	n.d.	n.d.	5	10	< 1
Gal	37	39	30	38	36	n.d.	n.d.	n.d.	30	39	59
4MeGlcA	20	24	25	21	n.d.	n.d.	n.d.	n.d.	14	7	8

[a]After treatment of *A* with proteinase K. [b]After treatment of *A* with Amberlite IR-120 (H^+) resin. [c]Derived from fractional precipitation with ammonium sulphate (see Scheme 1). [d]After chromatography of A60P, *etc.*, on DEAE-cellulose (see Fig. 1). [e]% N × 6.25. [f]Traces of Rha apparent in A1 and A2. [g]n.d. = Not determined.

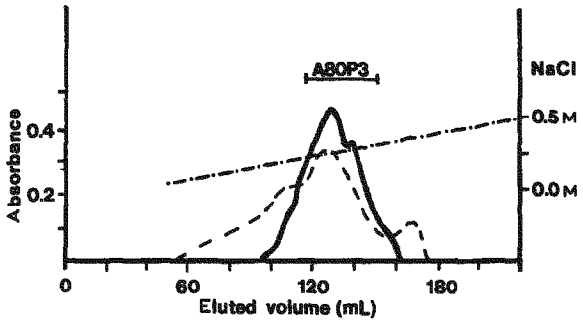


Fig. 1. Chromatography of A80P on DEAE-cellulose: carbohydrate (absorbance at 490 nm in phenol–H_2SO_4 assay), ——; absorbance at 220 nm, – – –; sodium chloride in Tris-HCl buffer (pH 8.6), —·—·—.

value dropped from −47° to −57° after 2 h, and p.c. showed the release of Ara and a trace of Gal. Thereafter, the gradual increase in the $[\alpha]_D$ value to −48° after 48 h was accompanied by a decrease in the Ara:Gal ratio in the hydrolysate. After 27 h, an aliquot (4 mL) was dialysed and freeze-dried, to give HA1 (9 mg) which contained Gal as the only neutral sugar component and was submitted to methylation analysis.

Isolation of aldobiouronic acids. — After *B* (15 g) had been heated in 0.5M H_2SO_4 at 100° for 6 h, fractionation of the hydrolysate by established methods[22] gave neutral and acidic fractions, the latter present as barium salts (2.36 g; ~50% carbohydrate). Chromatography (1:1:1 1-butanol–acetic acid–water), on a column (50 × 5 cm) of cellulose, of a portion (1 g) of the acidic fraction gave components I and II {R_{Gal} 0.93 and 0.86, solvent *B*; $[\alpha]_D$ +80° and +2°, respectively}. Samples of I and II were reduced in aqueous $NaBD_4$. Part of each product was deuteriomethylated and submitted to m.s. The remainder was methylated, hydrolysed, reduced ($NaBH_4$), acetylated, and analysed by g.l.c.–m.s.

Treatment of A *with proteolytic enzymes.* — (*a*) To a solution of *A* (400 mg) in 0.01M Tris-HCl buffer (25 mL, pH 7.8) containing 0.005M ethylenedinitrilotetraacetic acid (EDTA) and 0.5% SDS was added proteinase K (125 μL of a stock solution, 20 mg/mL in water, stored at −10°) and a few drops of toluene, and the solution was kept at 37°. The concentration of the amino acids produced, monitored by a ninhydrin method[23], became constant after ~4 h. The pH was then adjusted to 7.8 with M Tris-HCl buffer (pH 7.8) before further addition of proteinase K solution (125 μL). The whole procedure was repeated 6 times, the concentration of amino acids in the solution remaining unchanged after the fifth addition of proteinase K. Aliquots (0.1 mL) removed after the second and fifth treatments with the enzyme were examined by chromatography on Sepharose 4B (Fig. 2) in order to assess the extent of degradation. After the sixth addition of proteinase K, the solution was dialysed for 48 h against distilled water and freeze-dried to give PA (199 mg).

(*b*) A sample of A1 was treated with pronase as described above, to give a non-dialysable pronase-resistant product, PA1.

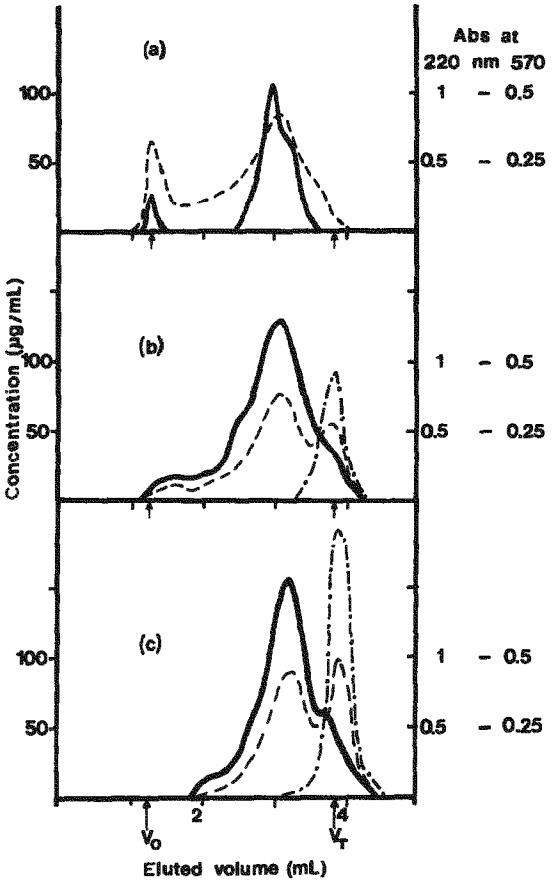


Fig. 2. Progress of digestion with proteinase K: (a) starting material (*A*), (b) products of 2 successive treatments with enzyme, (c) products of 5 successive treatments. Chromatography on Sepharose 4B, eluted with M NaCl: carbohydrate, ——; absorbance at 220 nm, ------; amino acids, –·–·–·–.

Alkaline hydrolysis. — A portion (100 mg) of PA1 was heated in 0.2M $Ba(OH)_2$ at 100° for 6 h. After neutralisation (aqueous 30% H_2SO_4) and centrifugation, the products recovered by freeze-drying the supernatant solution were fractionated by elution from a column (92 × 2 cm) of Bio-Gel P-2 (−400 mesh) with water at 60°. Fractions were monitored for carbohydrate[21] and for Hyp (Leach assay)[24].

RESULTS AND DISCUSSION

Exudates *A* and *B* were derived from specimens of *A. erioloba* from two different geographical locations. Since *B* was virtually insoluble in aqueous systems, including buffers containing 6M urea, a full comparison of *A* and *B* was not possible. However, as shown by the information in Table I and the amino acid compositions,

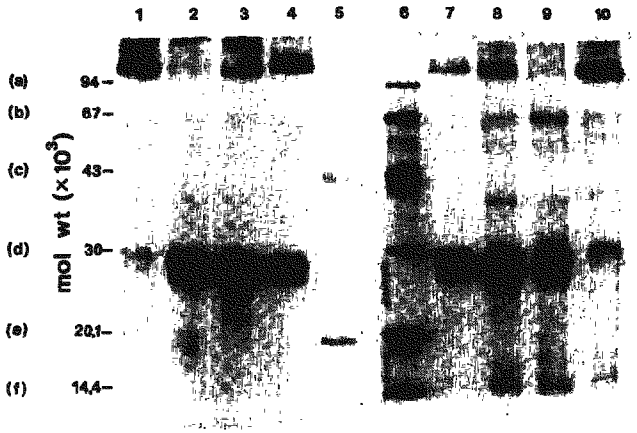
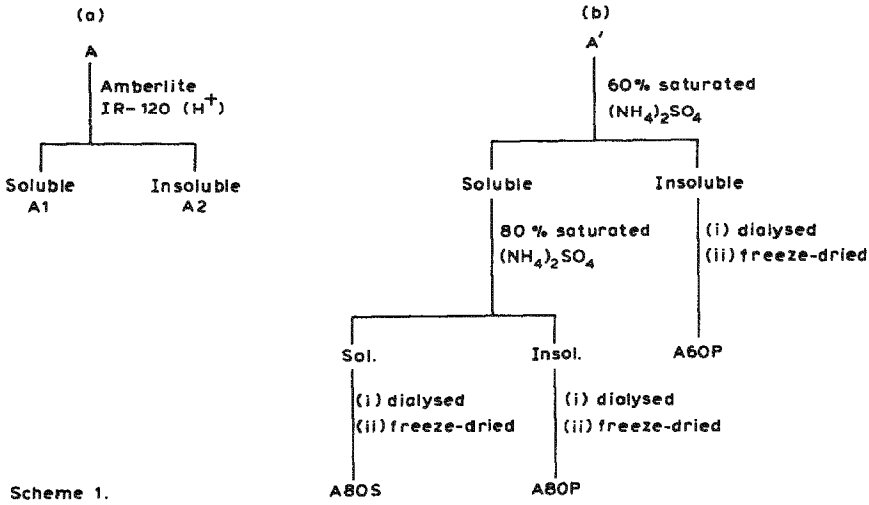


Fig. 3. SDS-PAGE of *A* (channels 1 and 10), A60P (channels 2 and 9), A80P (channels 3 and 8), A80S (channels 4 and 7), and protein standards (channels 5 and 6). Protein standards were: (a) phosphorylase b, (b) albumin, (c) ovalbumin, (d) carbonic anhydrase, (e) trypsin inhibitor, and (f) lactalbumin. Channels 1–5 were stained for carbohydrate, and 6–10 for protein.

they were closely similar in the proportions and composition of carbohydrate and protein. SDS-PAGE revealed that both *A* and *B* contained ranges of proteinaceous components having similar electrophoretic behaviour (shown for *A* in Fig. 3), their molecular weights being uncertain in the absence of suitable glycoprotein standards. On account of its solubility in water, *A* was used for studies of heterogeneity, vulnerability to proteolytic enzymes, and glycosyl-linkage composition, and *B*, of which a large quantity was available, for the isolation of products of controlled degradation.

The series of experiments summarised in Scheme 1 was undertaken in order to (*a*) demonstrate the presence of glycoproteins in the exudate and (*b*) establish the degree of heterogeneity of the preparation. SDS-PAGE showed (Fig. 3) that the exudate was a mixture of glycoproteins, migrating mainly as two groups of components with electrophoretic mobilities similar to those of carbonic anhydrase and phosphorylase b. This technique was used to monitor attempts at fractionation by precipitation with solutions of various degrees of saturation in ammonium sulphate and subsequent chromatography on DEAE-cellulose. The fractions A60P3, A80P3, and A80S3 so obtained differed in content of carbohydrate (Table I) but exhibited, in SDS-PAGE, a range of components similar to those in the parent material (Fig. 3). DEAE-cellulose chromatography (Fig. 1) did not provide a significant further fractionation, with the major fractions (*e.g.*, A80P3) being indistinguishable electrophoretically from the parent products.

Neither the exudate *A'* nor the fractions A60P and A80P exhibited haemagglutinating properties with human A, B, or O erythrocytes or rabbit erythrocytes, which indicates the absence of lectins, although the presence of lectins with affinity for sugar residues not present on erythrocyte surfaces, or not having haemagglutinating properties[25], cannot be ruled out.



Scheme 1. Fractionation of gum samples *A* and *A'* from *A. erioloba*.

De-ionisation of *A* with Amberlite IR-120 (H^+) resin caused precipitation of a high-protein fraction (A2; ~10% of *A*), having low proportions of Hyp (6.9 mol%) and Ser (8.9 mol%) among the amino acids, whereas the soluble fraction (A1) had enhanced proportions of Hyp and Ser (26.1 and 13.0 mol%, respectively). Fraction A2 contained a significant proportion of components adsorbed by hydroxyapatite and released on increasing the buffer concentration, whereas most of A1 was not adsorbed (Fig. 4), behaviour consistent with a higher degree of glycosylation in A1. In addition, exhaustive digestion of *A* with the proteolytic enzyme proteinase K afforded a non-dialysable product (PA) enriched in carbohydrate but having a protein component with increased contents of Hyp and Ser (34.3 and 16.4 mol% of amino acids) and electrophoretic behaviour similar to that of the major component in *A*. These results suggested the presence of a mixture of glycoproteins, varying in their degree of glycosylation as reflected in their proportions of the hydroxy amino acids.

Thus, the heterogeneity that is a common feature of glycoprotein preparations from plant sources[1] was manifested at each step of the fractionation attempts described here. In particular, the electrophoretic behaviour (Fig. 3) revealed numerous discrete components ("a continuous spectrum of closely related molecular species"[1]) which were not reflected in the chromatograms (Figs. 1, 2, and 4) upon which separation, on a practical scale for the structural work in hand, could be based. It was therefore decided to examine the structural features of the exudate as a whole.

Composition of the carbohydrate moiety. — The ^{1}H-n.m.r. spectrum of *A* contained, in addition to signals at δ 4.4–5.2 (anomeric protons) and at higher field (amino acid protons), a sharp singlet at δ 3.48 (OMe). Methanolysis of the glyco-

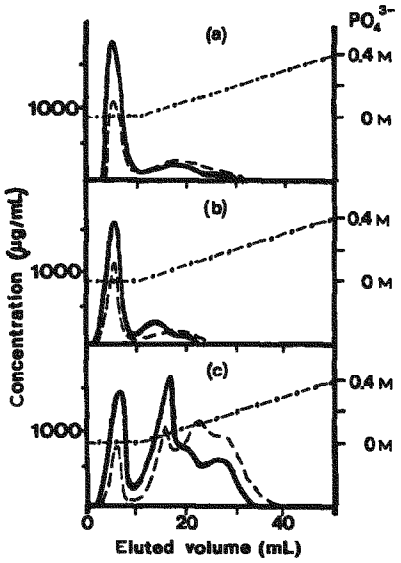
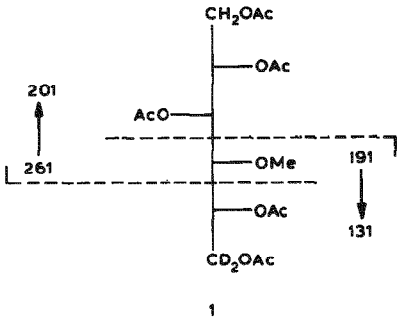


Fig. 4. Chromatography, on a column (10 × 0.5 cm) of hydroxyapatite, of (a) *A*, (b) A1, and (c) A2: carbohydrate, ——; absorbance at 220 nm, – – –; phosphate buffer (pH 7.4), —·—·—.

proteins, borodeuteride reduction of the methyl esters, hydrolysis, and conversion of the products into alditol acetates gave (g.l.c.–m.s.) 1,2,3,5,6-penta-*O*-acetyl-4-*O*-methyl[6,6-$^2H_2$]hexitol (**1**, see Scheme 2) as the only partially methylated derivative. From the g.l.c. retention-time (T_{Gal} 0.94, column *2*) of **1** and subsequent studies involving reduction and re-methylation of methylated A1 (Table II), the *gluco* configuration of **1** was deduced. Hence, **1** must have originated from 4-*O*-methylglucuronic acid. Glucuronic acid was absent, and Ara, Gal, and Man were present in the proportions given in Table I.

The modes of linkage of these sugars were established by analysis (Table II) of methylated A1. The role of the uronic acid was determined from studies of the



Scheme 2.

Scheme 2. Origin of the significant fragment ions in the mass spectrum of **1**.

TABLE II

METHYLATION ANALYSES FOR METHYLATED A1 (MA1) AND PRODUCTS DERIVED THEREFROM

Methyl ethers	*Proportions (mol %)*			
	MA1	*BMA1*[a]	*MRMA1*[b]	*MHA1*[c]
2,3,5-Ara[d]	31	38	30	—
3,5-Ara	5	< 1	9	—
2,3,4,6-Glc, 2,3,4,6-Man[e]	5[f]	14[g]	23[h]	—
2,3,4,6-Gal	6	39	8	30
2,3,6-Gal	14	4	11	27
2,3,4-Gal	12	5	11	31
2,4-Gal	6	—	8	12
Uronic acid	21[i]	—	—	n.d.

[a]Base-degraded, re-methylated MA1. [b]Methylated, reduced MA1. [c]Methylated HA1. [d]2,3,5-Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, *etc.* [e]Not resolved on OV-225. [f]Only Man present; see Table I. [g]Man content expected to rise to 6–7% on removal of uronic acid (21%); high figure may be due to co-eluting impurity (noted in related work on other polysaccharides). [h]Mainly 2,3,4,6-Glc, from terminal uronic acid. [i]From methanolysis.

base-degraded and re-methylated product (BMA1) and of the product (MRMA1) of carboxyl-reduction and re-methylation. In conformity with the results of methylation analysis, only small proportions of Ara and Gal were detected after periodate oxidation, borohydride reduction, and hydrolysis of *A*.

Two aldobiouronic acids (I and II), which were isolated after graded acid hydrolysis of *B*, had chromatographic mobilities similar to those of authentic **2** [α-4MeGlc*p*A-(1→4)-Gal] and **3** [β-4MeGlc*p*A-(1→6)-Gal]. Their structures were established unambiguously by the ^{1}H-n.m.r. (Table III), m.s. (Scheme 3), and methylation analysis data. The isolation of these aldobiouronic acids indicates that such entities occur as terminal units in significant proportion in the carbohydrate portion of the glycoprotein.

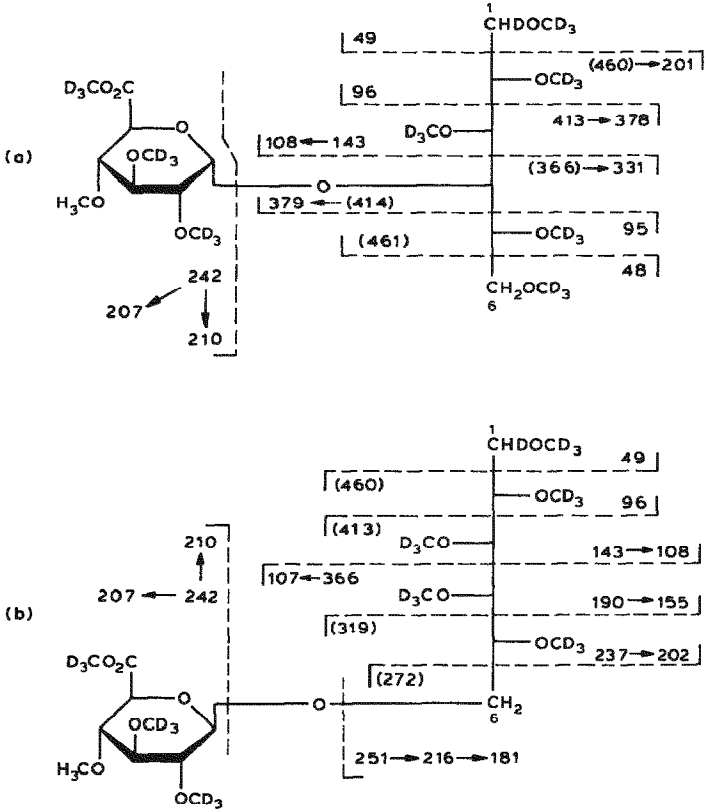
The high proportion of Hyp and Ser among the amino acids and the relative enrichment of these amino acids in the product of exhaustive digestion with proteolytic enzyme points both to their involvement in carbohydrate–protein covalent linkages and the ineffectiveness of the use of non-specific proteases in isolating small glycosides or glycopeptides incorporating the linkage region. The *O*-glycosylic linkage to Hyp is alkali-stable and its presence was therefore investigated by hydrolysis of the exudate glycoproteins in $Ba(OH)_2$ under conditions used in studies of related glycoproteins[4]. On partial fractionation of the products of one such treatment of PA1 on Bio-Gel P-2 (Fig. 5), the carbohydrate-containing components (PA1-I/IV), which were eluted at positions corresponding to mol. wts. of ~1500, ~950, ~450, and ~250 (using dextrans and standard oligosaccharides as calibrants), were all found to be associated with Hyp. Colorimetric assay showed that PA1-I and PA1-II contained uronic acid. It is possible to derive from Fig. 5 an approxi-

TABLE III

90-MHz ^{1}H-N.M.R. DATA[a] FOR I, II, REDUCED I, AND REDUCED II AT 80°

I			*Reduced I*			*II*[b]			*Reduced II*			
δ[c]	$J_{1,2}$[d]	*Integrated intensity*	*δ*	$J_{1,2}$	*Integrated intensity*	*δ*	$J_{1,2}$	*Integrated intensity*	*δ*	$J_{1,2}$	*Integrated intensity*	*Assignment*
5.31	2.8	0.33				5.25	3.4	0.5				H-1, α-Gal
4.96	3.4	1	5.07	3.4	1							H-1, α-4MeGlcA
4.60	7.3	0.66				4.56	7.1	1.5				H-1, β-Gal
						4.54	7.4		4.52	7.2	1	H-1, β-4MeGlcA
4.49	9.7	1										H-5, α-4MeGlcA
4.47	9.7											
3.48	*f*	3	3.48	*f*	3	3.50	*f*	3	3.50	*f*	3	-OMe 4MeGlcA

[a]Data for ring protons not included. [b]Data consistent with those of Peciar *et al.*[26]. [c]P.p.m. downfield from signal of Me_4Si. [d]In Hz. [e]$J_{4,5}$ value is tentative. [f]Singlet.



Scheme 3.

Scheme 3. M.s. fragmentation patterns for derivatives of (a) acid I and (b) acid II; *m/z* values in brackets were not observed. The peak with *m/z* 242 is from terminal uronic ester bearing one OMe by A_1 fragmentation[27] in each case. The spectrum of the derivative of I includes an ion *m/z* 314 which arises, as expected[28], from abJ_1 fragmentation.

mate distribution by weight of PA1-I/IV, the curve having been drawn up on the basis of calibration curves for galactose and arabinose, shown[29] to preponderate in the earlier and later fractions, respectively. The weight ratio was estimated as ~4:5:1:2 for PA1-I/IV, which, using the molecular weights indicated, represents a molar ratio of 2.5:5:2:8. The smaller products (PA1-III and -IV) together represent >50 mol % of the total.

The apparent ratio of carbohydrate to Hyp varies from ~11:1 for PA1-I to ~1.3:1 for PA1-IV. However, these values are undoubtedly low since Hyp residues in peptide linkages do not react[30,31] in the Leach assay[24] on the basis of which the curve was plotted, and further examination[29] of the products of alkaline hydrolysis of *A. erioloba* exudate itself showed that the conditions employed here, vigorous as they are, are insufficient to cleave completely the peptide linkages between extensively glycosylated Hyp residues. Consequently, contiguous glycosylated Hyp

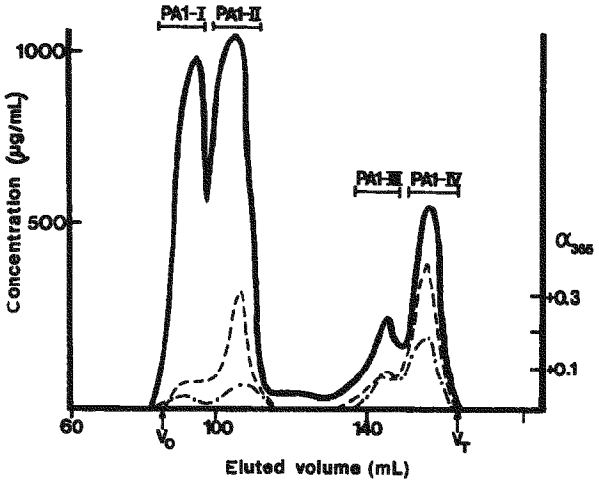


Fig. 5. Chromatography (Bio-Gel P-2) of the products of hydrolysis of PA1 with barium hydroxide: carbohydrate, ——; hydroxyproline, ------; optical rotation at 365 nm, -·-·-·-.

residues occur in many of the products obtained. Thus, the actual values of the carbohydrate:Hyp ratio for PA1-I/IV, and hence the average d.p. of the oligosaccharide chains, are lower than those suggested by the analytical data (Fig. 5). More-vigorous treatment of *A. erioloba* exudate by base[29] shifted the profile towards lower molecular weights, that of PA1-I being diminished and new components appearing within the 950–250 range. Further hydrolysis in base of the products, isolated by p.c. and studied by chemical and n.m.r. spectroscopic methods, proved that these could be degraded by peptide bond fission[29].

The results presented here indicate that the *A. erioloba* exudate has the following structural characteristics. (*a*) Little, if any, polysaccharide is present. The carbohydrate is distributed in the glycoprotein in the form of substituents on Hyp which have various short chain-lengths; the Hyp residues are in peptide chains. (*b*) All of the uronic acid, which is present solely as 4MeGlcA occurs (Table II) as non-reducing end groups, as do the Man, most of the Ara, and some of the Gal residues. The high proportion (~60 mol %) of the end groups and the very much lower proportion of branch points accords with the concept of glycosylation of Hyp residues with monosaccharides or short oligosaccharides. The molecular weights of the products of alkaline hydrolysis (Fig. 5) suggest the occurrence of contiguous glycosylated Hyp residues in many instances. (*c*) The oligosaccharide side-chains are mainly linear, but some branching is indicated by the 2,4-Me_2-Gal (~6 mol %) identified among the products of methylation analysis (Table II). (*d*) Base-catalysed degradation of MA1, followed by re-methylation, led to an increase in 2,3,4,6-Me_4-Gal and decrease in 2,3,6-Me_3-, 2,3,4-Me_3-, and 2,4-Me_2-Gal, and is consistent with the attachment of uronic acid mainly to either positions 4 or 6 of Gal residues. The corresponding aldobiouronic acid units have been isolated and

identified. (*e*) After removal of the Ara residues [all furanosyl; some (1→2)-linked, in addition to the terminal residues mentioned in (*b*)] by mild acid hydrolysis, the non-dialysable product (HA1) retained ~12% of the Gal residues as branch points, linked at positions 3 and 6, which indicates that none of the Ara residues are substituents of these units. The increased proportion of Gal end-groups in HA1, however, suggests that some Ara is linked through Gal in the oligosaccharide side-chains.

Thus, the exudate of *A. erioloba* has some unique structural features, notably the presence of uronic acid solely as 4MeGlcA and the high proportion of non-reducing end groups. The latter indicates an arrangement of the sugar residues as monosaccharide (mainly Ara) and short oligosaccharide side-chains (some branched) attached to Hyp residues in the peptide moiety. Further details of the structure of these side chains are given in a separate communication[29].

ACKNOWLEDGMENTS

We thank the C.S.I.R. and the University of Cape Town for generous grants, Ms. Charlene Steyn, Dr. E. P. Rybicki (Department of Microbiology), and Dr. E. H. Merrifield (Department of Clinical Science and Immunology) for advice and assistance, Miss Bridget Williamson (Mass Spectrometry Unit) and Dr. I. M. Moodie (Metabolic Research Unit, Tygerberg Hospital) for mass-spectrometric and amino acid analyses, respectively, and (the late) Professor E. A. C. L. E. Schelpe (Bolus Herbarium, University of Cape Town) for the authentication of *Acacia* species.

REFERENCES

1 A. E. CLARKE, R. L. ANDERSON, AND B. A. STONE, *Phytochemistry*, 18 (1979) 521–540.
2 R. R. SELVENDRAN AND M. A. O'NEILL, in F. A. LOEWUS AND W. TANNER (Eds.), *Encyclopaedia of Plant Physiology*, Vol. 13A, Springer-Verlag, Berlin, 1982, pp. 515–583.
3 G. B. FINCHER, B. A. STONE, AND A. E. CLARKE, *Annu. Rev. Plant Physiol.*, 34 (1983) 47–70.
4 D. ASHFORD, N. N. DESAI, A. K. ALLEN, A. NEUBERGER, M. A. O'NEILL, AND R. R. SELVENDRAN, *Biochem. J.*, 201 (1982) 199–208.
5 M.-C. VANDEVELDE AND J.-C. FENYO, *Carbohydr. Polym.*, 5 (1985) 251–273.
6 Y. AKIYAMA, S. EDA, AND K. KATŌ, *Agric. Biol. Chem.*, 48 (1984) 235–237.
7 D. M. W. ANDERSON, M. C. L. GILL, C. G. A. MCNAB, AND G. DE PINTO, *Phytochemistry*, 23 (1984) 1923–1926.
8 D. M. W. ANDERSON, M. C. L. GILL, A. M. JEFFREY, AND F. J. MCDOUGALL, *Phytochemistry*, 24 (1985) 71–75.
9 D. M. W. ANDERSON AND F. J. MCDOUGALL, *Phytochemistry*, 24 (1985) 1237–1240.
10 S. C. CHURMS AND A. M. STEPHEN, *Carbohydr. Res.*, 133 (1984) 105–123.
11 D. M. W. ANDERSON, J. C. K. FARQUHAR, AND C. G. A. MCNAB, *Phytochemistry*, 22 (1983) 2481–2484.
12 D. M. W. ANDERSON AND A. HENDRIE, *Carbohydr. Res.*, 20 (1971) 259–268.
13 S. C. CHURMS, E. H. MERRIFIELD, AND A. M. STEPHEN, *S. Afr. J. Chem.*, 36 (1983) 149–152.
14 D. W. GAMMON, S. C. CHURMS, AND A. M. STEPHEN, *Carbohydr. Res.*, 151 (1986) 135–146.
15 D. M. W. ANDERSON AND I. C. M. DEA, *Phytochemistry*, 8 (1969) 167–176.
16 A. M. STEPHEN AND S. C. CHURMS, *S. Afr. J. Chem.*, 39 (1986) 7–14.
17 W. F. DUDMAN, L.-E. FRANZÉN, J. E. DARVILL, M. MCNEIL, A. G. DARVILL, AND P. ALBERSHEIM, *Carbohydr. Res.*, 117 (1983) 141–156.

18 U. K. LAEMMLI, *Nature (London)*, 227 (1970) 680–685.
19 C. DUBRAY AND G. BEZARD, *Anal. Biochem.*, 119 (1982) 325–329.
20 H. LIS AND N. SHARON, *Methods Enzymol.*, 28 (1972) 360–365.
21 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
22 G. A. ADAMS, *Methods Carbohydr. Chem.*, 5 (1965) 269–276.
23 S. MOORE AND W. H. STEIN, *J. Biol. Chem.*, 211 (1954) 907–913.
24 A. A. LEACH, *Biochem. J.*, 74 (1960) 70.
25 J. A. CALLOW, *Curr. Adv. Plant Sci.*, 18 (1975) 181–193.
26 C. PECIAR, J. ALFÖLDI, R. PALOVČÍK, J. ROSÍK, AND J. KUBALA, *Chem. Zvesti*, 28 (1974) 83–85.
27 N. K. KOCHETKOV AND O. S. CHIZHOV, *Adv. Carbohydr. Chem. Biochem.*, 21 (1966) 39–93.
28 V. KOVÁČIK, V. MIHÁLOV, J. HIRSCH, AND P. KOVÁČ, *Biomed. Mass. Spectrom.*, 5 (1978) 136–145.
29 D. W. GAMMON AND A. M. STEPHEN, *Carbohydr. Res.*, 154 (1986) 289–295.
30 D. T. A. LAMPORT, *Nature (London)*, 216 (1967) 1322–1324.
31 D. T. A. LAMPORT, *Rec. Adv. Phytochem.*, 11 (1977) 79–115.