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Structural Studies on the Major Component of *Gladiolus* Style Mucilage, an Arabinogalactan-Protein

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The major component of the *Gladiolus* style mucilage was shown to be an arabinogalactan-protein. The arabinogalactan-protein was isolated from the style extract by affinity chromatography with tridacnin (the galactose-binding lectin from the clam *Tridacna maxima*) coupled to Sepharose 4B. The isolated arabinogalactan-protein represents 40% of the soluble style extract; it contains 90% (w/w) carbohydrate and 3% protein. The major monosaccharides of the carbohydrate component are galactose and arabinose, in the proportions 6:1. A component with a similar composition was also isolated from the crude extract by precipitation with the β-glucosyl artificial carbohydrate antigen. The protein moiety of the arabinogalactan-protein remained associated with the carbohydrate after chromatography in urea, and has high contents of serine, glutamic acid, aspartic acid, glycine and alanine. The arabinogalactan-protein is apparently chemically homogeneous; it eluted as a single symmetrical peak from Sepharose 4B, and three fractions collected across the peak were structurally similar. Ultracentrifugal studies showed it to be polydisperse in the mol.wt. range 150000–400000. The information obtained from methylation analyses, oxalic acid and enzymic hydrolyses is consistent with a model having a β1→3 galactan backbone, branched through C(O)6 to β1→6 galactan side chains. The arabinose is exclusively present as terminal α-L-arabinofuranosyl residues. Enzymic removal of the arabinose residues resulted in a marked decrease in solubility of the molecule. The localization of the arabinogalactan-protein in the mucilage of the style canal was demonstrated cytochemically. The possible roles of the arabinogalactan-protein in relation to recognition of compatible pollen and pollen-tube growth are discussed.

Fertilization in higher plants is preceded by capture of pollen by the receptive surface of the female stigma. If the pollen is compatible, it will germinate and a pollen tube will grow through the stylar canal, providing a conduit for the male gametes in their passage from the pollen grain to the ovary (Clarke & Knox, 1978).

We have examined the exudate that fills the *Gladiolus* style canal and have shown that the major component is an arabinogalactan-protein. Macromolecules containing galactose and arabinose are common constituents of plant tissues. They are major components of plant gums and have also been isolated from such diverse sources as seeds, leaves, roots, fruits, and both filtrates and cells of plant tissues in culture [for a review, see Clarke *et al.* (1979*a*)] and more recently have been detected in the stigmatic exudate of the lily *Lilium longiflorum* (Aspinall & Rosell, 1978).

Two approaches to the isolation of the arabinogalactans from plant extracts have been adopted in previous studies; classical fractionation of plant extracts (Aspinall, 1969) and precipitation with a class of dyes prepared by coupling diazotized 4-aminophenyl glycosides to phloroglucinol (Jermyn & Yeow, 1975). These dyes were first prepared by Yariv *et al.* (1962) as precipitating antigens for antibodies to glycoside determinants, and the β-glycosyl artificial carbohydrate antigen was shown to precipitate an arabinose-and-galactose-containing polymer from soya bean, jack bean and maize (Yariv *et al.*, 1967). Since then, this precipitation reaction has been widely used to isolate arabinogalactan-proteins from extracts of seeds of every taxonomic group of flowering plants, as well as leaf extracts and callus-culture filtrates [Jermyn & Yeow, 1975; Anderson *et al.*, 1977; and review by Clarke *et al.* (1979*a*)].

These dyes have also been used as cytochemical reagents for the localization of arabinogalactan-proteins in plant tissues (Clarke *et al.*, 1975; Clarke *et al.*, 1978). In seeds the arabinogalactan-proteins are concentrated in vesicles in the intercellular spaces, and in leaves and stems are present in secretory canals and other extracellular sites. We have used this reaction to demonstrate the presence of an arabinogalactan-protein in the style canal of *Gladiolus*; we

have also isolated this arabinogalactan-protein by precipitation with the β-glucosyl artificial carbohydrate antigen and by affinity chromatography using a galactose-binding lectin. We have then examined the structure of the isolated arabinogalactan-protein with a view to establishing how it may be involved in the recognition of compatible pollen and the nurture of the growing pollen tubes.

Experimental

Materials

Gladiolus gandavensis (gladioli) flowers were purchased locally. Sepharose 4B, Sepharose 6B, Sephadex G100, CNBr-activated Sepharose 4B and the dextrans were from Pharmacia Fine Chemicals, Uppsala, Sweden. Peanut lectin was from IBF, Clichy, France. The α-L-arabinofuranosidase, purified from Pectinol R-10, was a gift from Professor M. Neukom (Swiss Federal Institute of Technology, Zurich, Switzerland) (Neukom *et al.*, 1967). The *Larix* (larch) galactan was purchased from Koch–Light Laboratories, Colnbrook, Bucks., U.K. β-D-galactopyranosyl-1→3-D-arabinose was from Pfanstiehl Laboratories, Waukegan, IL, U.S.A. The g.l.c. packing material, 3% SP2340 on 100/120 Supelcoport, was obtained from Supelco Inc., Bellefonte, PA, U.S.A. *m*-Phenylphenol was obtained from ICN Pharmaceuticals, Plainview, NY. U.S.A. All other chemicals were of the highest purity available.

The J539 myeloma was a gift from Professor M. Potter (National Institutes of Health, Bethesda, MD, U.S.A.) and was passaged in mice by Dr. R. Ceredig (Walter and Eliza Hall Institute, Melbourne, Vic., Australia).

Methods

Preparation of style extract from Gladiolus gandavensis. Cut flowers of *Gladiolus gandavensis* were maintained in 0.01% 8-hydroxyquinoline citrate and 1% sucrose solution. Mature pistils were collected 24h after flower opening, the stigmas removed and the remaining styles were homogenized in a Waring blender with 0.05M-Tris/HCl/0.15M-NaCl/1mM-$CaCl_2$, pH7.4, at 4°C (10g of style fresh weight extracted per 100ml of buffer). The extract was centrifuged at 25000*g* for 30min at 4°C, and the supernatant dialysed against water and freeze-dried. This material did not redissolve completely. The undissolved material was removed by centrifugation before application of the supernatant to the chromatography columns.

Gel chromatography on Sepharose 4B. Gel chromatography was performed on a column (91cm × 1.5cm) of Sepharose 4B equilibrated in 0.02M-sodium phosphate buffer, pH7.0, containing 0.15M-NaCl and 0.02% NaN_3. The flow rate of the column was 12ml/h, and 4ml fractions were collected. The column was calibrated for molecular weight with dextran standards of weight-average molecular weights ($\bar{M}_w$) 41000, 268000, 478000 and 2000000. The apparent molecular weight of the style arabinogalactan-proteins was estimated from a plot of the partition coefficient ($K_{av.}$) against log $\bar{M}_w$ of the standards.

Purification of the galactose-binding lectin (tridacnin) from the clam Tridacna maxima. Specimens of *Tridacna maxima* were collected from the Great Barrier Reef, Queensland, Australia. The clams were transported frozen to the laboratory where they were thawed, opened, and the haemolymph (body fluid) collected. The haemolymph was dialysed against water and freeze-dried. Tridacnin was purified by affinity chromatography on acid-treated Sepharose 6B by the method of Baldo & Uhlenbruck (1975). The purified lectin was dialysed, freeze-dried and stored at 4°C (yield approx. 0.5mg of purified lectin per ml of haemolymph).

Affinity chromatography of Gladiolus style extract on tridacnin–Sepharose. Tridacnin was coupled to Sepharose 4B as previously described (Gleeson *et al.*, 1979). *Gladiolus* style extract was dissolved in 0.15M-NaCl containing 0.01M-$CaCl_2$ and loaded directly on to the tridacnin–Sepharose 4B column. The column dimensions were 3.5cm × 1cm, or 17cm × 1cm when larger quantities of bound material were required. The conditions of chromatography were as previously described (Gleeson *et al.*, 1979).

Artificial carbohydrate antigens.‡ The β-D-glucopyranosyl and α-D-galactopyranosyl artificial carbohydrate antigens of Yariv *et al.* (1962) were a gift from Dr. M. A. Jermyn, Division of Protein Chemistry, C.S.I.R.O., Parkville, Vic., Australia, and were prepared by coupling the diazotized 4-aminophenyl glycosides with phloroglucinol (Yariv *et al.*, 1962). These are highly coloured glycosyl phenylazo dyes having the structural formula shown in Fig. 1, but exist in a highly aggregated form in solution (Woods *et al.*, 1978).

β-Glucosyl artificial carbohydrate antigen-binding assay. This was performed as described by Jermyn & Yeow (1975). The final artificial-carbohydrate-antigen arabinogalactan-protein precipitate was redissolved in 5M-sodium thiocyanate and the A_{405} measured (Clarke *et al.*, 1978).

Carbohydrate determination. Total carbohydrate was determined colorimetrically by the phenol/sulphuric acid method (Dubois *et al.*, 1956), with galactose as standard, or was calculated by addition of amounts of individual monosaccharides determined by g.l.c.

Uronic acid determination. Uronic acids were

‡ These molecules will be referred to here as β-glucosyl and α-galactosyl artificial carbohydrate antigens. They have also been referred to elsewhere as 'glycosyl Yariv antigens' (Jermyn & Yeow, 1975; Anderson *et al.*, 1977).

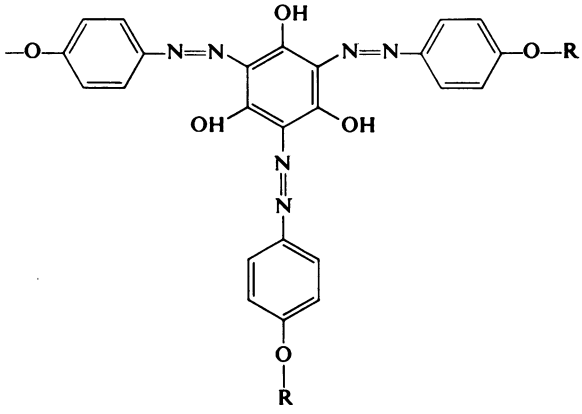


Fig. 1. *Structural formula of the artificial carbohydrate antigen*
R=β-glucosyl or α-galactosyl residue.

determined colorimetrically by the method of Blumenkrantz & Hansen (1973), with glucuronic acid as standard.

Monosaccharide composition. Samples (1–2mg) were hydrolysed in 2ml of 2.5M-trifluoroacetic acid at 100°C for 2h in a sealed tube under nitrogen. After hydrolysis the acid was removed on a rotary evaporator, and the hydrolysate was then reduced with sodium borohydride and acetylated (Albersheim *et al.*, 1967). The resulting alditol acetate derivatives were separated by g.l.c. on a 1.85m×4mm column of 3% SP2340 on 100/120 Supelcoport, in a Hewlett–Packard model 5710A gas chromatograph. The chromatography was conducted isothermally at 215°C with an N_2-carrier-gas flow rate of 60ml/min.

Amino acid analysis. Samples were hydrolysed by boiling under reflux in 6M-HCl for 24h under nitrogen. The high-dilution technique of Pusztai & Morgan (1963) was used (protein concentration less than 0.01%). Insoluble material was produced during the hydrolysis of the complex between the style extract and the β-glucosyl artificial carbohydrate antigen. This insoluble material, which was probably derived from phenols released from the artificial carbodrate antigen, was removed from the hydrolysate by filtration.

Haemagglutination assay. Haemagglutination was performed with trypsin-treated human erythrocytes by the method of Bowles & Kauss (1975). Doubling dilutions of the samples were made, and the lowest dilution at which agglutination was detectable was taken as the end point and expressed as 2^n.

Paper chromatography. Paper chromatography was performed on Whatman no. 3 paper in ethyl acetate/pyridine/water (8:2:1, by vol.). The chromatograms were developed for 18h and then stained for reducing sugar with silver nitrate (Trevelyan *et al.*, 1950).

Gel diffusion. Gel diffusion was performed in 5% (w/v) Davis gelatine containing 0.15M-NaCl and 0.02% (w/v) NaN_3, for 24h at 37°C in a humidity chamber, then examined for the presence of precipitation lines. Gelatine was used, in preference to agar, as the immunoglobulin A protein of the J539 myeloma was found to bind to the agar matrix.

Cellulose acetate electrophoresis. Cellulose acetate electrophoresis of the *Gladiolus* style arabinogalactan-protein was carried out in Tris/barbital/sodium barbital buffer, *I* 0.05, pH8.8, by using a Beckman Microzone electrophoresis apparatus, for 45min at 4mA. The membrane was stained with β-glycosyl artificial carbohydrate antigen (1mg/ml in 0.15M-NaCl) for 10min and destained in 0.15M-NaCl.

Methylation analysis. Samples (1.8–5.0mg) were dissolved in 4ml of dimethyl sulphoxide and methylated three times by the method of Hakomori (1964). The methyl sulphinyl anion was prepared as described by Conrad (1972). The permethylated samples were dialysed exhaustively against water, freeze-dried, and hydrolysed with formic acid followed by sulphuric acid, as described by Lindberg *et al.* (1972). The partially methylated sugars were reduced and acetylated (Albersheim *et al.*, 1967), and the resulting alditol acetates separated by g.l.c. on a 1.85m×2mm glass column of 3% OV 225 on Chrom WHP (80/100 mesh).

The retention times of the separated components were measured relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. The components were tentatively identified by reference to the retention times published by Lonngren & Pilotti (1976) and by comparison with material prepared at the same time by methylation of larch galactan and the disaccharide β-D-galactoypyranosyl-(1→3)-D-arabinose.

Partial acid hydrolysis of the arabinogalactan-protein. The *Gladiolus* style arabinogalactan-protein was dissolved in 1ml of 12.5mM-oxalic acid and heated at 100°C for 5h (Bishop, 1957). The hydrolysate was cooled to 16°C and made 80% (v/v) with respect to ethanol. The precipitated material was washed thoroughly with 80% ethanol and freeze-dried. This fraction was examined by methylation analysis. The 80%-ethanol-soluble fraction and washings of the precipitate were pooled, a small portion removed for analysis by paper chromatography, and the remainder evaporated to dryness. This fraction was redissolved in water and divided into halves; one half was reduced and acetylated directly for analysis of the free monosaccharides by g.l.c., whereas the other half was further hydrolysed in 2.5M-trifluoroacetic acid at 100°C for 2h, then reduced and acetylated for determination of total monosaccharides by g.l.c.

Enzymic hydrolysis of the arabinogalactan-protein. *Gladiolus* style arabinogalactan-protein (7.3mg) in

7.3ml of 0.05M-sodium acetate buffer, pH4.8, was incubated with a quantity of α-L-arabinofuranosidase that produced complete hydrolysis. A sample (150μl) was removed for determination of reducing sugars (Nelson, 1944; Somogyi, 1952). A further portion of enzyme was added and the mixture incubated for a further 24h at 30°C, after which a 150μl sample was removed for measurement of total reducing sugars. The sequence was repeated a third time. There was no increase in reducing-sugar content after the second addition of enzyme, and the reaction was assumed to be complete. The reaction mixture was concentrated and made 80% (v/v) with respect to ethanol at 15°C. The precipitated material was washed thoroughly in 80% ethanol, redissolved in water, dialysed exhaustively against water, and freeze-dried (yield 5.8mg). The monosaccharide composition of this fraction was determined, and methylation analysis was also carried out. The 80%-ethanol-soluble fraction and the washing were pooled and evaporated to dryness. This ethanol-soluble fraction was reduced and acetylated directly for the determination of free monosaccharides.

Ultracentrifugation. The molecular weight of the style arabinogalactan-protein was determined by the miniscus-depletion-sedimentation-equilibrium method of Yphantis (1964) by using a Beckman model E ultracentrifuge equipped with interference optics. The sample (0.04% in 0.01M-sodium phosphate buffer, pH7.0, containing 0.15M-NaCl) was centrifuged at 10000rev./min (r6.5cm) for 70h, followed by a further 24h at 8000rev./min at 20°C. Sedimentation-velocity experiments were performed at 60000 rev./min, at 20°C, with scans at 8min intervals by schlieren optics.

Viscosity. Viscosity measurements were made (in 0.1M-NaCl) at 25.0°C in a Cannon Ubbelohde viscometer. The reduced viscosity ($\eta_{sp.}/c$) was determined at various concentrations of arabinogalactan-protein and the intrinsic viscosity ([η]) calculated.

Solubility. Solubility was measured by suspending sufficient sample in a known volume of water at 20°C for 15min with intermittent shakings, so that some material remained undissolved. The undissolved material was removed by centrifugation at 30000g for 60min and the supernatant was freeze-dried and weighed. The solubility of the sample was taken as the weight of material per ml of supernatant.

Polarimetry. Optical rotation was measured in a Stanley photoelectric polarimeter at 20°C in a 1ml jacketed cell.

Cytochemical localization of the Gladiolus style arabinogalactan-protein. Intact *Gladiolus* pistils were removed from mature flowers, embedded and frozen at −30°C in a gel containing 15% (w/v) gelatine and 2% (w/v) glycerol. Blocks were cut containing segments of the frozen style. Transverse sections of the style were cut in a cryostat (Cryocut; American Optical Corp.), air-dried on microscope slides and stained with β-glucosyl artificial carbohydrate antigen (1mg/ml in 0.15M-NaCl) for 10min, followed by two washes with 0.15M-NaCl, then examined by bright-field microscopy. As a control of the staining specificity, sections were treated in a similar way with the α-galactosyl artificial carbohydrate antigen.

Results

Fractionation of Gladiolus gandavensis style extract and identification of the major component

Gel chromatography. Gel chromatography of the

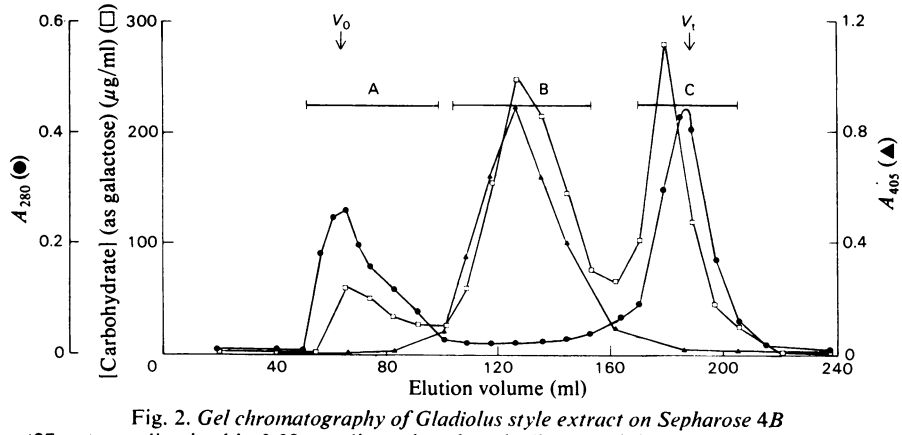


Fig. 2. *Gel chromatography of Gladiolus style extract on Sepharose 4B*

Style extract (27mg) was dissolved in 0.02M-sodium phosphate buffer containing 0.15M-NaCl, pH7.0, and loaded on a column (91cm × 1.5cm) of Sepharose 4B, which was run with a flow rate of 12ml/h. Fractions were analysed for carbohydrate (□), for the capacity to bind to the β-glucosyl artificial carbohydrate antigen (A_{405}, ▲), and the A_{280} was measured (●). Fractions were pooled as indicated (A, B and C). The arrows show V_0 (void volume) and V_t (total volume).

style extract on Sepharose 4B gave the profile shown in Fig. 2. Three carbohydrate peaks were obtained and pooled individually as indicated. The monosaccharide analyses of the unfractionated style extract and of the three fractions are given in Table 1. The major monosaccharides of the style extract were arabinose and galactose. Glucose and xylose were present as minor constituents, and traces of fucose and rhamnose were also detected. The three fractions obtained by Sepharose 4B chromatography had marked differences in their total carbohydrate content as well as their monosaccharide composition. Fraction B, in which the highest proportion of the applied carbohydrate was recovered, had the highest carbohydrate content, namely 60.7%; the monosaccharides of this fraction were galactose and arabinose in the ratio 6.1:1, and all of the recovered arabinose from the starting material was in this fraction. On the other hand, both fractions A and C had relatively low carbohydrate contents (5.2 and 4.0% respectively). Fraction A contained galactose and xylose in the proportions 1:2, and all the xylose recovered was found in this fraction. Glucose was the sole monosaccharide component of fraction C, and no glucose was associated with the other fractions. Only 47.3% of the carbohydrate applied to the column was recovered in the three fractions, and no further material was recovered even after exhaustive buffer washing of the column. Two other parameters, the ability to bind to the β-glucosyl artificial carbohydrate antigen and the haemagglutinating activity were measured. The capacity to bind the β-glucosyl artficial carbohydrate antigen paralleled the carbohydrate profile of fraction B, and was not associated with either fractions A or C (Fig. 2). Haemagglutination activity was detected in fraction A (titre 2^5) and fraction B (titre 2^2, both at 10 mg/ml). The haemagglutination titre of concanavalin A, used at the same concentration as the fractions, was 2^{15}.

Affinity chromatography. Interaction of the *Gladiolus* style extract with galactose-binding macromolecules. The style extract gave single precipitin bands in double-diffusion tests with the galactose-binding peanut lectin, tridacnin, and also with the β-glucosyl artificial carbohydrate antigen. Two precipitin bands were observed with the galactose-binding immunoglobulin A protein of the mouse myeloma J539.

Affinity chromatography of style extract on tridacnin–Sepharose 4B. The material of the style extract that interacted with tridacnin was isolated by affinity chromatography on tridacnin–Sepharose (Fig. 3). The bound material was eluted in a calcium-free solution and represented approximately half of the total material recovered, and accounted for 75% of the total carbohydrate applied to the column. The unbound fraction represented 10% of the carbohydrate applied; therefore 15% of the applied carbohydrate was not recovered from the column.

The tridacnin-bound fraction was further subjected to Sephadex G-100 chromatography in 8 M-urea, to remove any non-covalently bound material. The carbohydrate applied was recovered in a single symmetrical peak that emerged at the void volume. All the subsequent analyses of the tridacnin-bound material were performed on material that had been subjected to this Sephadex G-100-chromatography step.

The monosaccharide analyses of the bound and unbound fractions from tridacnin–Sepharose are given in Table 2. The fraction bound to tridacnin–Sepharose contained 90% carbohydrate, and the major monosaccharides were galactose and arabinose, in a ratio of 6:1 with trace amounts of glucose and rhamnose also present. There was also 3% protein associated with this fraction. The unbound fraction contained 15.6% carbohydrate, of which xylose was the major monosaccharide, and galactose, arabinose, glucose and rhamnose were also present. All the recovered xylose, rhamnose and glucose of the crude extract were present in this fraction.

The capacity to bind to and precipitate the β-glucosyl artificial carbohydrate antigen paralleled the elution profile of the tridacnin-bound material (Fig. 3), and the unbound material had no detectable capacity to bind to the β-glucosyl artificial carbohydrate antigen. Thus the tridacnin-bound material resembles fraction B obtained by conventional Sepharose 4B chromatography in both the capacity to bind to the β-glucosyl artificial carbohydrate antigen and in the galactose/arabinose ratio (6:1) of the isolated fraction. The analyses indicate the arabinogalactan-protein nature of this material.

Table 1. *Monosaccharide composition of Gladiolus style extract and fractions of style extract obtained by gel chromatography on Sepharose 4B*

	Monosaccharide composition (%, by wt., of total carbohydrate)			
		Style fractions from Sepharose 4B chromatography:		
Monosaccharide	Crude Style extract	A	B	C
Galactose	65.1	33.7	85.9	Trace
Arabinose	17.2	0	14.1	0
Glucose	8.2	Trace	Trace	100
Xylose	7.7	66.3	0	0
Rhamnose	1.8	0	0	0
Fucose	Trace	0	0	0
Galactose/arabinose ratio			6.1:1.0	
Carbohydrate in sample (%)	45.9	5.2	60.7	4.0

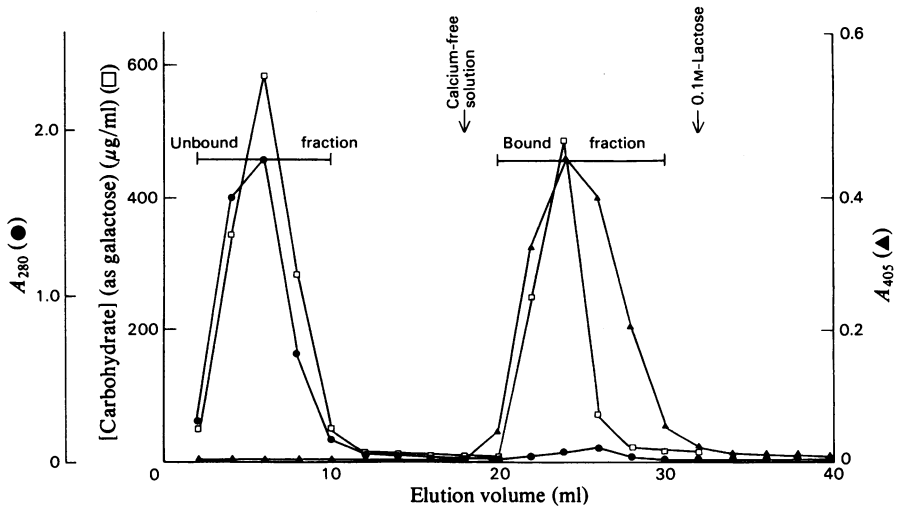


Fig. 3. *Affinity chromatography of Gladiolus style extract on tridacnin–Sepharose 4B*

Style extract (10mg) was dissolved in 0.15M-NaCl containing 0.01M-$CaCl_2$ and loaded on a column (3.5cm×1.0cm) of tridacnin–Sepharose 4B that had been equilibrated in 0.15M-NaCl containing 0.01M-$CaCl_2$. The column was washed with the same solution to remove all non-bound material; the arrows indicate the position at which elution with calcium-free solution and 0.1M-lactose respectively were started. Fractions were analysed for carbohydrate (□), the capacity to bind to the β-glucosyl artificial carbohydrate antigen (A_{405}, ▲), and the A_{280} was measured (●). The unbound fraction and the bound fraction were pooled as indicated.

Table 2. *Monosaccharide composition of Gladiolus style fractions prepared by tridacnin–Sepharose chromatography and by precipitation with β-glucosyl artificial carbohydrate antigen*

	Monosaccharide composition (%, by wt., of total carbohydrate)			
		Tridacnin–Sepharose chromatography		Material precipitated by the β-glucosyl artificial carbohydrate antigen†
Monosaccharide	Crude style extract	Unbound fraction	Bound fraction*	
Galactose	65.1	17.7	85.8	63.4
Arabinose	17.2	17.1	14.2	13.5
Glucose	8.2	22.9	Trace	†
Xylose	7.7	38.3	0	0
Rhamnose	1.8	3.9	Trace	Trace
Fucose	Trace	Trace	0	0
Galactose/arabinose ratio			6.0:1.0	4.7:1.0
Carbohydrate in sample (%)	45.9	15.6	90	
Protein in sample (%)			3.0	1.6

* Results are the means for three injections on each of five samples obtained from separate chromatographic runs of the affinity column.

† Results are the means for three injections of two separate preparations precipitated by the β-glucosyl artificial carbohydrate antigen. The precipitate was analysed directly. The amount of glucose arising from the style material could not be assessed under these conditions of precipitation as the precipitating antigen contributed a high proportion of glucose to the analysis.

Analysis of the style arabinogalactan-protein

A comparative analysis of the style arabinogalactan-protein prepared by tridacnin–Sepharose chromatography and by precipitation with β-glucosyl artificial carbohydrate antigen is given in Table 2.

The monosaccharide analyses of the two preparations were similar in that galactose was the major monosaccharide and that galactose and arabinose were present in approximately the same proportions in both preparations. For the material prepared by

precipitation with the β-glucosyl artificial carbohydrate antigen, it was not possible to calculate the total carbohydrate originating from the style material, as it was associated with a high proportion of glucose derived from the precipitating antigen.

The material prepared by affinity chromatography had only trace amounts of glucose; previously we reported the presence of significant, but variable, amounts of glucose associated with the tridacnin-purified material (Gleeson *et al.*, 1979). Methylation analysis of these preparations showed the glucose to be linked either by a 1→4 or 1→6 linkage, suggesting that it may originate from starch associated with arabinogalactan-protein. By exhaustively washing the affinity column after application of the crude extract, the amount of glucose in the preparation could be decreased to trace amounts.

The amino acid analyses of the arabinogalactan-protein purified by the two methods are similar (Table 3). Both preparations had high contents of serine, glutamic acid, aspartic acid, glycine and alanine and low contents of cystine, methionine and hydroxyproline. The tridacnin-purified arabinogalactan-protein also had 0.9% uronic acid.

Methylation analysis of the arabinogalactan-protein, prepared by tridacnin–Sepharose chromatography, is shown in Table 4. All the arabinose is terminal and in the furanose form. The galactan is composed of both 1→3-and 1→6-linked galactopyranosyl residues and is highly branched as indicated by the high content (38.6%) of 1,3,6-linked galactopyranosyl residues.

The homogeneity of a sample of the arabinogalactan-protein isolated by tridacnin affinity chromatography was examined by Sepharose 4B chromatography (Fig. 4) and by analysis of the material eluting at the leading edge, the centre and the tailing edge of the single peak obtained. The three fractions are very similar with respect to glycosyl units and linkage types (Table 4) and the analyses of these fractions are similar to that of the whole preparation (Table 4), indicating chemical homogeneity of the arabinogalactan moiety of the isolated arabinogalactan-protein.

The isolated arabinogalactan-protein gave single precipitin bands against tridacnin and peanut lectin, and two precipitin bands against the immunoglobulin A protein of the J539 myeloma.

Partial hydrolysis of the isolated style arabinogalactan-protein

Enzymic hydrolysis. The style arabinogalactan-protein, prepared by tridacnin–Sepharose 4B chromatography, was treated with α-L-arabinofuranosidase under conditions that gave maximum release of reducing sugar. All the arabinose residues from the arabinogalactan-protein were removed under these conditions, indicating that all the arabinose residues are terminal and in the α-L-arabinofuranosyl form, and confirming the methylation analysis of the arabinogalactan-protein. The methylation analysis of the galactan-protein recovered after enzymic hydrolysis is shown in Table 5. The galactan-protein retains its ability to bind to the β-glucosyl artificial carbohydrate antigen, but its solubility in water is markedly lower than that of the native molecule (Table 6).

Table 3. *Amino acid composition of Gladiolus style arabinogalactan-protein*

	Composition (mol% of recovered amino acids)	
Isolation method	... Affinity chromatography on tridacnin–Sepharose	Precipitation by β-glucosyl artificial carbohydrate antigen
Amino acid		
Lys	3.2	5.0
His	2.9	2.0
Arg	3.3	5.5
Asp	9.6	10.4
Thr	6.0	6.8
Ser	14.7	9.0
Glu	10.1	7.1
Pro	3.0	4.4
Gly	14.0	9.3
Ala	8.0	9.4
½-CyS	1.2	1.2
Val	4.6	6.2
Met	0.6	0.9
Ile	3.5	4.7
Leu	3.8	5.9
Tyr	3.0	3.8
Phe	3.4	4.8
Hyp	<0.9	<3.0
Orn*	3.0	0
GlcN	1.1	1.2
Protein as percentage of sample	3.0	1.6

* Tentative identification

Mild acid hydrolysis. A sample of arabinogalactan-protein, prepared by tridacnin–Sepharose chromatography, was hydrolysed with 12.5 mM-oxalic acid at 100°C for 5 h and the hydrolysate was fractionated by precipitation with 80% ethanol. Examination of the 80%-ethanol-soluble fraction by paper chromatography showed the presence of free arabinose and free galactose as well as unresolved reducing material remaining close to the origin. Monosaccharide analysis of the 80%-ethanol-soluble fraction showed that all the arabinose of the starting material was recovered in this fraction as the free monosaccharide and a low proportion (7.4%) of the total galactose was recovered as the free monosaccharide. The total monosaccharide composition of this sample, determined after complete hydrolysis, showed that 25% of the galactose of the initial sample was present in

Table 4. *Methylation analysis of the style arabinogalactan-protein*

The style arabinogalactan-protein was prepared by tridacnin–Sepharose chromatography. A sample of this material was subjected to gel chromatography on Sepharose 4B and three fractions were prepared as indicated in Fig. 4. These fractions and the whole arabinogalactan-protein were methylated, hydrolysed, and the resultant partially methylated sugars reduced, converted into alditol acetates and examined by g.l.c. For full details, see the text. Retention times are expressed relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol. The peak areas obtained were converted into mole values by use of the molar response factors given by Sweet *et al.* (1975).

				Linkage composition (mol %)			
					Fractions prepared of the arabinogalactan-protein by gel chromatography on Sepharose 4B (Fig. 4)		
Peak no.	Relative retention time	Tentative identification	Linkage type	Arabinogalactan-protein purified by tridacnin–Sepharose chromatography	I	II	III
1	0.43	1,4-Di-*O*-acetyl-2,3,5-tri-*O*-methyl-L-arabinitol	L-Arabinofuranosyl-(1→	12.9	14.5	13.6	14.5
2	1.01	1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol	D-Glucopyranosyl-(1→	Trace	3.1	4.1	3.7
3	1.19	1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol	D-Galactopyranosyl-(1→	29.4	26.0	27.3	25.0
4	2.05	1,3,5-Tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-galactitol	→3)-*O*-D-Galactopyranosyl-(1→	13.7	13.9	11.4	14.2
5	2.89	1,5,6-Tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-galactitol	→6)-*O*-D-Galactopyranosyl-(1→	5.6	3.2	4.4	5.9
6	5.10	1,3,5,6-Tetra-*O*-acetyl-2,4-di-*O*-methyl-D-galactitol	→3,6)-*O*-D-Galactopyranosyl-(1→	38.6	39.0	39.2	36.8

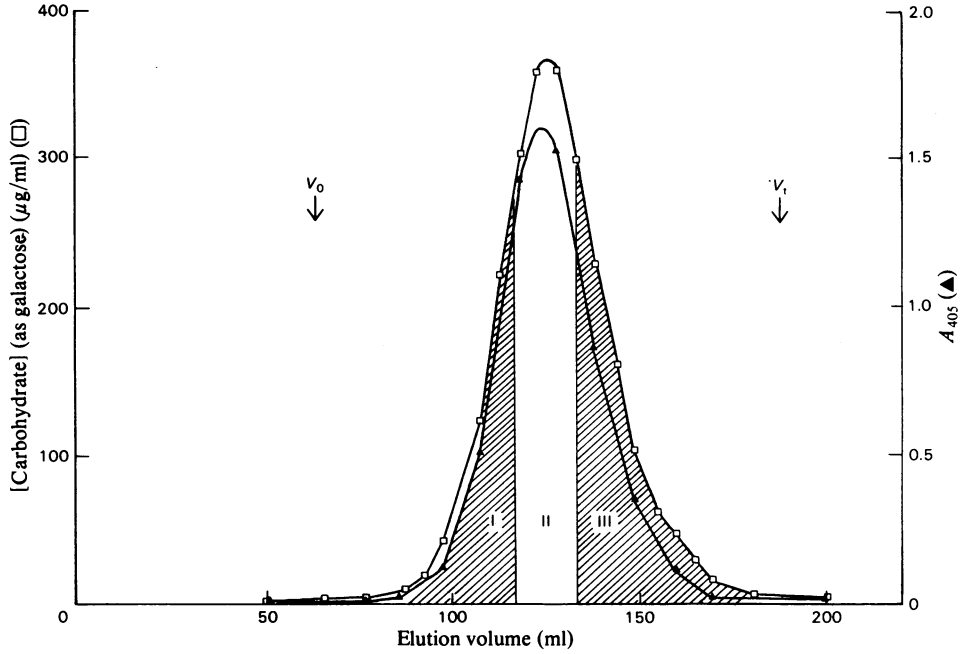


Fig. 4. *Gel chromatography of the tridacnin–Sepharose 4B-bound fraction on Sepharose 4B*

The tridacnin–Sepharose 4B-bound style fraction (14mg) was dissolved in 0.02M-sodium phosphate buffer, containing 0.15% NaCl, pH7.0, and loaded on to a column (91cm×1.5cm) of Sepharose 4B, equilibrated in the same buffer, which was run with a flow rate of 12ml/h. Fractions were analysed for carbohydrate (□) and the capacity to bind the β-glucosyl artificial carbohydrate antigen (A_{405}, ▲). The arrows show V_0 (void volume) and V_t (total volume). Three fractions were collected from the single peak as indicated.

this fraction. The 80%-ethanol-insoluble fraction contained only galactose and trace amounts of glucose. Methylation analysis of this galactan is shown in Table 5.

Physical properties of the style arabinogalactan-protein

The purified arabinogalactan-protein elutes as a single symmetrical peak of apparent mol.wt. 260000 when examined by gel chromatography on Sepharose 4B (Fig. 4). Cellulose acetate electrophoresis of the purified arabinogalactan-protein showed only one component after staining with β-glucosyl artificial carbohydrate antigen. This component was positively charged at pH8.8, but the nature of this positive charge has not been elucidated. The molecular-weight, solubility, viscosity and optical-rotation data for the purified arabinogalactan-protein are shown in Table 6. The behaviour of the arabinogalactan-protein at sedimentation equilibrium indicates that it is polydisperse in the mol.wt. range 150000–400000. There was an even distribution of molecular-weight species within this range. During velocity sedimentation, a symmetrical boundary formed initially, but then spread into a broad area, indicating again the polydisperse nature of the material. The specific optical rotation ($[\alpha]_D^{20}$) of the native molecule was −10 (*c* 0.2 in water). The solubility of the arabinogalactan-protein was > 5mg/ml in water. However, after treatment with the α-L-arabinofuranosidase the molecule has a solubility of only 1mg/ml. This low solubility made it impracticable to measure the specific rotation of the derived galactan.

Localization of the arabinogalactan protein in Gladiolus style

The mucilage in the style canal of *Gladiolus* stains intensely with the β-glucosyl artificial carbohydrate antigen, as do the secretory cells lining the canal (Plate 1*a*). No staining was observed with the α-galactosyl artificial carbohydrate antigen (Plate 1*b*).

Discussion

Isolation of the major style component of Gladiolus

The major component of the style homogenate could be isolated either by conventional gel chromatography (Fig. 2) or more simply by affinity chromato-

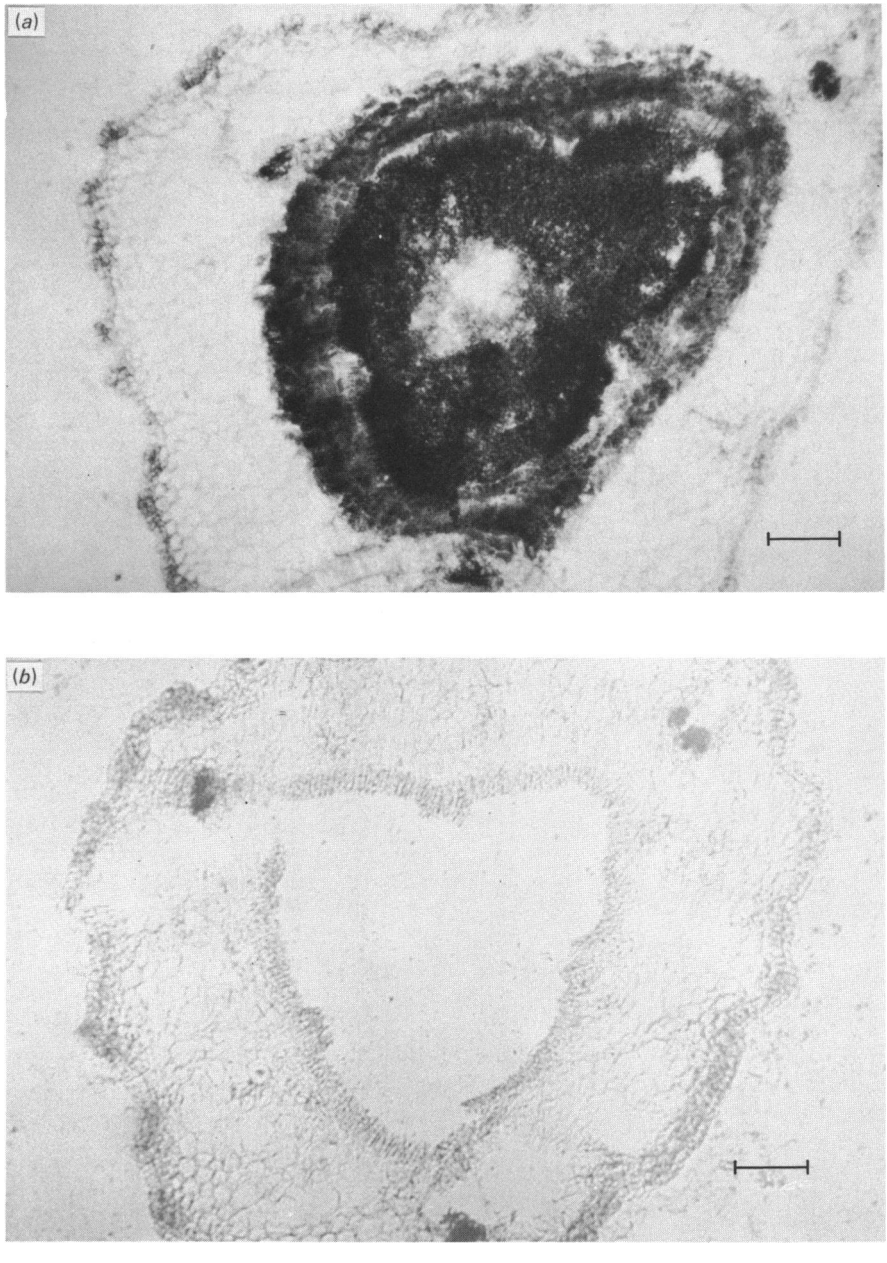
Table 5. *Methylation analyses of the α-L-arabinofuranosidase-treated and oxalic acid-treated arabinogalactan-protein*

The treated arabinogalactan-proteins were obtained as described in the text. The samples were methylated, hydrolysed and the resultant partially methylated sugar reduced and converted into alditol acetates and examined by g.l.c. Retention times (R_t) are expressed relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-glucitol. The peak areas obtained were converted into relative mol values by the use of the molar response factors given by Sweet *et al.* (1975).

				Linkage composition (mol %)		
Peak no.	R_t	Tentative identification	Linkage type	Native arabino-galactan	α-L-Arabino-furanosidase-treated arabino-galactan-protein*	Oxalic acid-treated arabino-galactan-protein†
1	0.43	1,4-Di-*O*-acetyl-2,3,5-tri-*O*-methyl-L-arabinitol	L-Arabinofuranosyl-(1→	12.9	Trace	Trace
2	0.70	?		0	0	0.6
3	0.84	?		0	0	1.0
4	1.01	1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol	D-Glucopyranosyl-(1→	Trace	Trace	1.2
5	1.19	1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol	D-Galactopyranosyl-(1→	29.4	39.4	35.0
6	2.04	1,3,5-Tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-galactitol	→3)-*O*-D-Galactopyranosyl-(1→	13.7	9.2	11.7
7	2.89	1,5,6-Tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-galactitol	→6)-*O*-D-Galactopyranosyl-(1→	5.6	13.8	18.4
8	5.1	1,3,5,6-Tetra-*O*-acetyl-2,4-di-*O*-methyl-D-galactitol	→3,6)-*O*-D-Galactopyranosyl-(1→	38.6	37.6	32.4

* There was also an additional peak with an R_t value of 8.7, which accounted for 40% of the recovered material. It is probably a monomethylated galactitol derivative, and as it was not found in the methylated native molecule, it probably resulted from either undermethylation of the sample or hydrolysis of the methylated sample.

† There were two additional peaks with R_t values of 8.7 and 10.2, which accounted for 9.5 and 14.3% respectively of the recovered material. These are probably monomethylated galactitol derivatives, and as they were not found in the methylated native molecule, they probably resulted from either undermethylation of the sample or hydrolysis of the methylated sample.



EXPLANATION OF PLATE 1

Localization of the arabinogalactan-protein in the Gladiolus style

A transverse section of the *Gladiolus* style was stained for the arabinogalactan-protein with β-glucosyl artificial carbohydrate antigen (1 mg/ml in 0.15 M-NaCl) for 10 min, followed by two washes with 0.15 M-NaCl (*a*). As a control of the staining specificity, a section was treated in a similar way with α-galactosyl artificial carbohydrate antigen (*b*). The bar represents 150 μm.

Table 6. *Physicochemical parameters of the arabinogalactan-protein from Gladiolus style*

Parameter	Value
Optical rotation ($[\alpha]_D^{20}$)	−10 (*c* 0.2 in water)
Intrinsic viscosity (η)	0.09 dl/g
Molecular weight	
(*a*) Gel filtration ($M_{app.}$)	260000
(*b*) Sedimentation equilibrium	
8000 rev./min (*r* 6.5 cm)	180000–450000
10000 rev./min (*r* 6.5 cm)	150000–400000
Solubility in water (S^{20})	
Native arabinogalactan-protein	>5 mg/ml
Galactan-protein*	1 mg/ml

* Obtained from α-L-arabinofuranosidase treatment of the native arabinogalactan-protein.

graphy (Fig. 3). The monosaccharide analyses of the material isolated by these methods were very similar, galactose and arabinose being present in both samples in the ratio 6:1. The components isolated by these two methods both reacted to the same extent with the β-glucosyl artificial carbohydrate antigen (Figs. 2 and 4). Material precipitated from a style homogenate with the β-glucosyl artificial carbohydrate antigen gave an analysis closely similar to that of the major components isolated chromatographically (Table 2), indicating that both components are arabinogalactan-proteins. The basis of the interaction between the β-glucosyl artificial carbohydrate antigen and the arabinogalactan-protein is not completely understood. Whether the binding involves the carbohydrate of the protein component of the arabinogalactan-protein, or perhaps both components, has not been established.

Although only isolation of a glycopeptide would confirm that the protein is covalently linked to the carbohydrate component, the finding that carbohydrate and protein are present in the same ratio, whether the material is isolated by precipitation with the artificial carbohydrate antigen or by affinity chromatography followed by gel chromatography in the presence of urea, indicates close carbohydrate–protein association.

The arabinogalactan-protein isolated represented about 50% of the total material recovered from the affinity column and 40% of the total material applied to the column. The loss of carbohydrate material during both gel (Fig. 2) and affinity chromatography was significant (55 and 15% respectively), and no further material could be recovered in spite of exhaustive washing with buffer, or 0.1 M-lactose.

Of the minor components detected during the fractionation procedure by gel chromatography, the high-molecular-weight component A (Fig. 2) was noteworthy since it was associated with haemagglutinating activity. The presence of haemagglutinins in pistil extracts of *Primula* has been demonstrated, and they have been implicated in pollen recognition and the control of pollen-tube growth (Golynskaya *et al.*, 1976). In the course of the present study we confirmed that haemagglutinins were present in pistil extracts of *Primula*, and demonstrated their presence in *Gladiolus* pistil extracts; however, whether they are present in the secretion or in an intracellular site was not established, and their role, like that of plant lectins in general, remains obscure.

Structure of the arabinogalactan-protein isolated from Gladiolus

The isolated arabinogalactan-protein preparation was apparently chemically homogeneous; it eluted as a single symmetrical peak from Sepharose 4B, and three fractions collected from the leading edge, peak and tailing edge of the elution profile were apparently structurally identical (Table 4). However, equilibrium-sedimentation analysis showed that the preparation was polydisperse within the mol.wt. range 150000–400000. Chemical homogeneity of a population of molecules of different sizes is compatible with suggestion that biosynthesis of these secreted proteoglycans might involve assembly of saccharide blocks, the number of blocks added to the primer molecule being variable. The polysaccharide components of certain *Acacia* and *Larix* gums have been shown to contain a range of discrete components with molecular weights which are multiples of a 6000-mol.wt. component (Churms *et al.*, 1977, 1978).

Methylation analysis of the isolated arabinogalactan-protein indicated that all the galactose is present as either terminal 1→3-, 1→6- or 1,3,6-linked galactosyl residues, and that all arabinose is present in the furanose form as terminal non-reducing groups. Table 7 shows the approximate molar ratios of these linkage types that were found in the arabinogalactan-protein. This structural information is compatible with a model in which a 1→3-linked galactan backbone is branched through C(O)6 to side branches of 1→6-linked galactosyl residues, some of which carry the terminal arabinofuranoside residues (Fig. 5). However, this is not the only model that is compatible

Table 7. *Molar ratios of glycosyl linkages in the Gladiolus style arabinogalactan-protein*
The molar ratios have been calculated, to the nearest whole number, from the methylation data (Table 5).

Linkage type	Molar ratio (native arabinogalactan-protein)	Difference in relative molar ratio	
		After α-L-arabinofuranosidase treatment	After oxalic acid treatment
Terminal arabinosyl	2	−2	−2
Terminal galactosyl	6	+1	0
1→3-Linked galactosyl	3	−1	−1
1→6-Linked galactosyl	1	+1	+2
1,3,6-Linked galactosyl	7	−1	−2

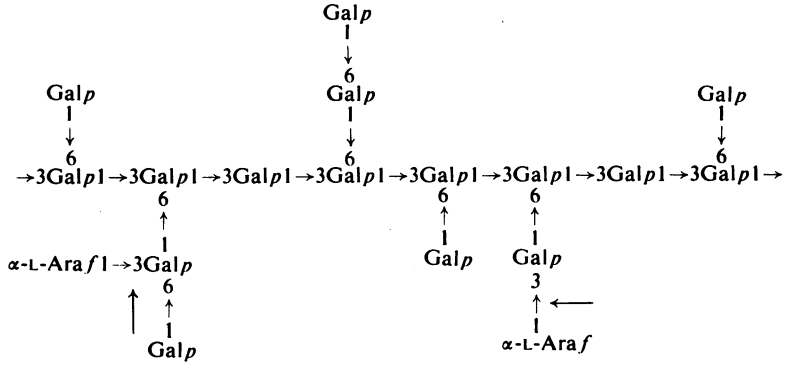


Fig. 5. *A proposed model of the style arabinogalactan-protein*
The arrows indicate the possible positions of linkage cleavage by α-L-arabinofuranosidase.

with the data, and others such as a branch-on-branch type structure or one involving mixed-linked 1→3-, 1→6-linked galactan chains can also be envisaged. The galactopyranosyl residues are probably in the β-anomeric configuration, as the native arabinogalactan-protein has a low negative optical rotation indicative of a high content of β-linked residues (Timell, 1965). Also the arabinogalactan-protein was found to interact with β-galactose-specific binding proteins, tridacnin and peanut lectins, as well as the immunoglobulin A protein of the J539 myeloma.

Enzymic hydrolysis with α-L-arabinofuranosidase confirmed the terminal position of the arabinosyl residues and established the α-confirguration of these linkages. Comparison of the methylation analysis of the arabinogalactan-protein before and after enzymic hydrolysis showed that release of terminal arabinosyl residues was accompanied by an equimolar increase in the sum total of terminal galactosyl residues and 1→6-linked galactosyl residues. There was a corresponding decrease in the sum total of 1→3-linked galactosyl residues and 1,3,6-linked galactosyl residues (Table 7). Fig. 5 shows possible positions of the terminal arabinosyl residues compatible with this information. The increase in terminal galactosyl residues, corresponding to one-half of the molar loss of arabinose, together with an equivalent decrease in the 1→3-linked galactosyl residues suggests that one-half of the arabinose residues are 1→3-linked at the end of the galactan chains (Fig. 5). The increase in 1→6-linked galactosyl residues, again corresponding to one-half of the molar loss of arabinose, together with the decrease in the 1,3,6-linked galactosyl residues, indicates that the remaining arabinose residues are 1→3-linked to an internal 1→6-linked galactosyl residue (Fig. 5).

Methylation analysis of the enzyme-treated arabinogalactan-protein was difficult because of the decreased solubility of the treated material and complete methylation was possibly not achieved. Nevertheless, the results are internally consistent as there is a molar equivalence between total terminal mono-

saccharide residues and branch points. Moreover, the results are compatible with the analyses of native arabinogalactan-protein.

A similar comparison of the methylation analyses of the native arabinogalactan-protein and the galactan recovered after mild acid hydrolysis indicated, as expected, that all the susceptible terminal arabinofuranoside residues were released. In addition to changes in linkage composition resulting from loss of terminal arabinose, other changes manifested by loss of 1,3,6-galactosyl and terminal galactosyl residues and gain of 1→6-linked galactosyl residues were noted (Table 7). This pattern can not be accounted for by cleavage of a single linkage type, and, as hydrolysis even under these mild conditions also released both galactose and galactosyl oligosaccharides, it is likely that the molecule is degraded under these conditions both at the side chains and at the backbone. Fragmentation of interior galactan chains by mild acid hydrolysis has been noted previously with the *Lolium* arabinogalactan-protein (Anderson *et al.*, 1977) and probably occurred with a number of *Acacia* gum arabinogalactans (Anderson *et al.*, 1968*a*; Anderson & Munro, 1969) as well as the wheat endosperm arabinogalactan-peptide (Fincher *et al.*, 1974), since substantial decreases in molecular weight of these polymers were observed after mild acid hydrolysis.

The isolated style arabinogalactan-protein had a high content of the hydroxy amino acid serine, as well as glycine, aspartic and glutamic acids. The serine content is noteworthy, since this amino acid has been shown to be involved in glycopeptide linkages in other plant proteoglycans and glycoproteins that contain arabinose and galactose. A galactosyl-*O*-serine linkage has been demonstrated in the proteoglycan isolated from *Cannabis sativa* leaves (Hillstead & Wold, 1977), the potato lectin (Allen *et al.*, 1978) and the cell-wall glycoprotein extensin (Lamport *et al.*, 1973). The low hydroxyproline content in the style arabinogalactan-protein was unexpected (Table 3): this amino acid is characteristically high in arabinogalactan-proteins isolated from many plant tissues by precipitation with the β-glucosyl artificial carbohydrate antigen (Jermyn & Yeow, 1975; Anderson *et al.*, 1977), and is involved in the carbohydrate–protein linkage in a number of characterized plant macromolecules. For example a hydroxyproline–galactose linkage has been defined in an arabinogalactan peptide from wheat endosperm (McNamara & Stone, 1978) and in the cell-wall glycoprotein of the unicellular alga *Chlamydomonas* (Miller *et al.*, 1972), and a similar linkage has been implicated in the arabinogalactan-protein secreted into the medium by suspension-cultured *Acer pseudoplatanus* (sycamore) cells (Pope, 1977). Hydroxyproline has also been shown to be involved in a linkage through arabinose in the potato lectin (Allen *et al.*, 1978) and in extensin (Lamport, 1977); however, as all the arabinose of the style arabinogalactan-protein is in the terminal position, a linkage involving arabinose and hydroxyproline in the style arabinogalactan-protein is unlikely. The significance of the unidentified base in the style arabinogalactan-protein that cochromatographed with ornithine is not known, but a similar basic amino acid has been noted in other arabinogalactan-proteins (Jermyn & Yeow, 1975; Anderson *et al.*, 1977) as well as in the potato lectin (Allen *et al.*, 1978).

The composition of the carbohydrate component of *Gladiolus* style arabinogalactan-protein is very similar to that of the major component of the receptive stigma surface of the same plant (Clarke *et al.*, 1979*b*). The stigma surface has been shown to contain an arabinogalactan accounting for at least 20% of the total material that can be washed from the mature stigma surface. This material contained galactose and arabinose as the major monosaccharides in the ratio 3.8:1. However, neither the linkage composition nor the association with protein has been established for the stigma surface component. The mucilage of both the stigma and style of *Lilium longiflorum* have also been examined during the course of a study of nutrition of pollen-tube growth (Labarca & Loewus, 1972, 1973). The mucilage from both sites contained galactose and arabinose as the major monosaccharides; methylation analysis of the stigma mucilage (Aspinall & Rosell, 1978) showed that arabinose, although representing a higher proportion of total monosaccharide than found for *Gladiolus* style arabinogalactan-protein, was present as terminal residues, and the galactose was present as 1→3, 1→6-, and 1,3,6-linked residues. However, as well as these components, both rhamnose and glucuronic acid were present in the preparation. The fractions used in these (Aspinall & Rosell, 1978) studies were probably mixtures of carbohydrate-containing macromolecules but it seems likely that there is a major component of the *Lilium longiflorum* stigma and style exudate that is closely related chemically to that of the *Gladiolus* stigma and style.

The arabinogalactans or arabinogalactan-proteins associated with the female reproductive tissues of both plants are related to a widely distributed class of proteoglycans that have a general structure based on a 1→3-linked galactan backbone with variations in the degree of branching, the length of the side chains and the degree of substitution of the chains by terminal arabinosyl residues. Their association with protein has only been established in a few instances. Members of this group have been described from gymnosperm wood, gum exudates (especially of *Acacia* spp.), as well as from cultured callus cells and whole tissues. The chemistry and biology of this group of proteoglycans has recently been reviewed (Clarke *et al.*, 1979*a*).

Biological role of the style arabinogalactan-protein

The question as to the possible role of the style arabinogalactan-protein now arises: it may be required as a physical support for the growing pollen tube; it may also provide exogenous nutrient for the biosynthesis of the primary cellulosic wall of the pollen tube. The pollen grain at maturity has extensive starch reserves, which are mobilized during pollen-tube growth, but whether they are sufficient to support the entire growth of the tube is not established. Labarca & Loewus (1972) have presented evidence that growing pollen tubes of *Lilium longiflorum* can incorporate exogenous substrate into the walls; the conclusion was based on experiments in which a labelled preparation of a stigma exudate was injected directly into the canals of excised styles. Not only was this material incorporated into the pollen-tube walls, but the style-canal mucilage was shown to be degraded to material of a lower molecular weight during pollen-tube growth. Scanning electron micrographs of fractured *Gladiolus* styles after pollination show an apparent depletion of the mucilage as pollen-tube growth progresses (Clarke *et al.*, 1977); we have also shown that *Gladiolus* pollen-wall preparations are enzymically active, having the capacity to degrade isolated style arabinogalactan-protein with release of arabinose and galactose (P. A. Gleeson, S. Harrison & A. E. Clarke, unpublished observations). These observations are consistent with the possibliity of a nutritive role for the style arabinogalactan-protein in *Gladiolus*.

Although both general supportive and nutritive roles are possible for the style arabinogalactan-protein, it is also possible that it performs a more specific function in information exchange between the growing pollen tube carrying the sperm cells and the female tissues of the style. The open-branched structure of the arabinogalactan-protein is particularly suited to interactions with other macromolecules. Interactions between polysaccharides, especially in regions of regular linkage sequences, are known (Rees, 1975); also arabinogalactans have been shown to bind to a number of flavonol glycosides (Jermyn, 1978) and specifically to various lectins. Again these types of interaction could constitute a primary event in information exchange at the pollen-tube surface.

There is at present insufficient information available to assign a particular role to the arabinogalactans of the female reproductive tissues of *Gladiolus*; however the structural features of the major component of the style canal described in the present paper could form a basis for further studies aimed at defining their role in the sequence of events of pollination and fertilization in flowering plants.

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