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ARABINOGALACTANS OF SEXUAL AND SOMATIC TISSUES OF *GLADIOLUS* AND *LILIUM*

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Abstract—Arabinogalactans are present in extracts of the sexual and somatic tissues of two monocotyledons, *Gladiolus* and *Lilium*. The arabinogalactans precipitated from plant extracts by the β-glucosyl artificial antigen (Yariv antigen) show differences in the proportions of the major monosaccharides, galactose and arabinose. *Gladiolus* stigma and style preparations contain a single arabinogalactan component, but the other tissues examined contained at least two distinct groups of arabinogalactans. These groups can be separated electrophoretically or by lectin affinity chromatography.

INTRODUCTION

Arabinogalactans are widely distributed in plant tissues. The group of arabino 3,6 galactans (Aspinall Type II [1]) are major components of many gums and have been found in such diverse tissues as roots, seeds, leaves, fruits and both filtrates and cells of plant tissues in culture [2]. More recently, they have been described from the female reproductive tissues of *Gladiolus* [3,4], as well as the stigma exudate of *Lilium longiflorum* [5,6]. The arabinogalactans from this group appear to have a common structure based on a 1,3β galactan backbone to which are attached side branches of 1,6-linked galactosyl residues linked through C(O)6, some of which terminate in arabinosyl residues [2]. Often these arabinogalactans are covalently associated with protein [2]. Recently, a large number of representatives from this group have been isolated [7–9] by a precipitation reaction with the β-glucosyl artificial carbohydrate antigens described by Yariv [10]. These artificial carbohydrate antigens are glucosyl phenyl azo dyes and they precipitate arabinogalactans if the glucosyl groups of the artificial antigen are in the β configuration, but not if they are in the α configuration. The precise nature of the regions of the arabinogalactan and the dye involved in the reaction is not known: however, the reaction has proved to be most useful both in the isolation and cytochemical localisation of this group of proteoglycans.

The arabinogalactans have been implicated in such diverse functions as cell–cell adhesion, nutrition of growing pollen tubes and as a response to microbial infection [2]. It has also been suggested that they may be markers of identity in plant tissues, variation in identity being expressed in the terminal sequences of the saccharide chains [2]. As a basis for exploring their biological role we have examined the arabinogalactans from a number of different tissues of two plants, *Gladiolus* and *Lilium*. We have used the interaction with the β-glucosyl artificial antigen to detect and isolate the arabinogalactans; we have then examined their electrophoretic behaviour as well as their capacity to interact with specific galactose-binding proteins.

RESULTS AND DISCUSSION

(a) *Detection and characterization of arabinogalactans from sexual and somatic tissues of* Gladiolus *and* Lilium

Gel diffusion against the β-glucosyl artificial antigen. Plant tissue extracts can be screened for the presence of arabinogalactans by gel diffusion of the extract against the β-glucosyl artificial antigen [7,9]. The presence of arabinogalactans is indicated by formation of a red precipitin band in the gel. When examined in this way the *Gladiolus* stigma surface preparation and the style, leaf and petal extracts all gave a single precipitin band.

Extracts of *Lilium longiflorum* tissues behaved similarly: stigma surface, style, leaf and petal all gave single precipitin bands with the β-glucosyl artificial antigen. The aqueous extracts of somatic tissues of both species were prepared from ethanol-treated tissues. This precaution was taken as arabinogalactans from other plant tissues were only detected with the β-glucosyl antigen if the tissues were initially extracted with ethanol [9]. The alcohol treatment apparently extracted flavanol glycosides which may be complexed to the arabinogalactans in whole tissues. The precipitation reaction between arabinogalactans and the β-glucosyl artificial antigen can be inhibited by flavanol glycosides [11], reflecting perhaps structural similarities of the artificial antigen and the flavanol glycosides [11]. These screening tests show that arabinogalactans are present in the sexual and somatic tissues of both *Gladiolus* and *Lilium*.

Electrophoretic behaviour. Specific staining of arabinogalactans with the β-glucosyl artificial antigen can also

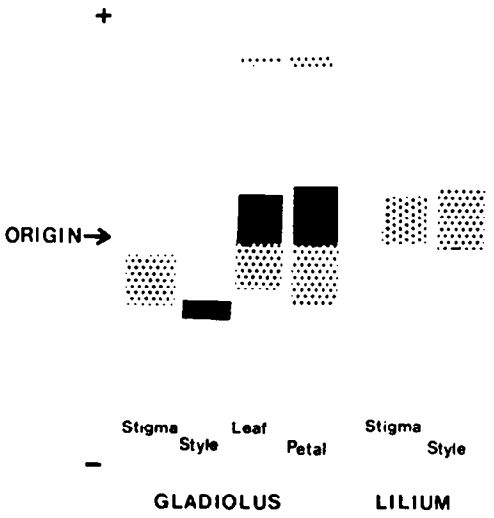

Fig. 1. Cellulose acetate electrophoresis of *Gladiolus* and *Lilium* extracts. Extracts were applied to a cellulose acetate membrane and electrophoresis was carried out in Tris–barbital–sodium barbital buffer, pH 8.8, for 20 min at 4 mA. The membrane was stained with β-glucosyl artificial antigen (1 mg/ml in 0.15 M NaCl) for 10 min and destained in 0.15 M NaCl. The concentrations of the extracts were as follows: *Gladiolus* stigma and style 10 mg/ml; *Gladiolus* leaf and petal 50 mg/ml; *Lilium* stigma 10 mg/ml; *Lilium* style 25 mg/ml.

be used for their visualisation on cellulose acetate membranes after electrophoresis of tissue extracts. Cellulose acetate electrophoresis of the *Gladiolus* stigma, style, leaf and petal extracts at pH 8.8, followed by staining with the β-glucosyl artificial antigen, gave the patterns shown in Fig. 1. The stigma and style material both gave a single positively charged band; these bands moved to approximately the same extent under the experimental conditions, but differed in that the stigma material stained in a more diffuse band than the style material. The leaf and petal material behaved quite differently: both had two major components, one negatively charged and one positively charged, and in addition a minor highly negatively charged component.

Extracts prepared from *Lilium* stigma and style were also examined electrophoretically; both gave single diffuse negatively charged bands which remained close to the origin (Fig. 1). Electrophoresis on cellulose acetate membranes required loading a very small sample volume. Extracts from *Lilium* leaf and petal were extremely viscous and could not be concentrated to the small loading volume required for electrophoresis on cellulose acetate membranes.

As well as confirming the presence of arabinogalactans in the tissue extracts examined, electrophoresis showed that *Gladiolus* somatic tissues contain two distinct groups of arabinogalactans, both of which interact with the β-glucosyl artificial antigen. The basis for the charge differences of these two groups is not known at present. The heterogeneity of arabinogalactans from somatic tissues is also reflected in their interaction with galactosyl-binding lectins (see Section (b)).

Charge heterogeneity of arabinogalactans from other plant extracts has also been observed by cellulose acetate electrophoresis [7]; and in one case, *Alocasia macrorhiza* leaf extract, electrophoretically different components were isolated and shown to be arabinogalactan-proteins which differed in the composition of both the carbohydrate and protein components [12].

Monosaccharide analysis. The precipitation reaction of arabinogalactans with the β-glucosyl antigen was also used for their isolation from *Gladiolus* style, leaf and petal and from *Lilium* stigma and style. The monosaccharide analyses of these isolated arabinogalactans is given in Table 1. An analysis of the *Gladiolus* stigma and style arabinogalactans prepared by tridacnin-affinity chromatography is also included for comparison.

All the analyses show galactose and arabinose as the major monosaccharides, confirming the arabinogalactan nature of the material which interacts with the β-glucosyl artificial antigen. However, there is variation in the relative amounts of galactose and arabinose present as shown by the galactose:arabinose ratios. For example, *Gladiolus* stigma and style arabinogalactans contain appreciably more galactose (76 and 86%, respectively) than those from leaf and petal (69 and 64%, respectively), or from *Lilium* stigma and style (64 and 64%, respectively). Rhamnose was also present in significant amounts in *Gladiolus* leaf and petal and the *Lilium* stigma and style arabinogalactans but not in the *Gladiolus* stigma and style arabinogalactans (Table 1).

Analyses of *Gladiolus* style arabinogalactan prepared by either tridacnin affinity chromatography or precipitation with the β-glucosyl artificial antigen are very similar, indicating that similar material was isolated by both methods. It was not practical to isolate the *Gladiolus* stigma arabinogalactan by both methods, as the yield of the stigma surface extract is very low (0.2 mg/100 stigmas).

An arabinogalactan from the *Lilium* stigma exudate has also been described by Aspinall and Rosell [6]. This was prepared by ion-exchange chromatography of the *Lilium* stigma exudate; the neutral monosaccharide composition of this fraction [5] is similar to that prepared by precipitation of the *Lilium* stigma surface extract with the β-glucosyl artificial antigen (Table 1).

Arabinogalactans have previously been isolated from a variety of plant tissues and exudates and it has been suggested that variation in this class of proteoglycans, expressed in the proportions of monosaccharides, may have some taxonomic significance [13]. Certainly, comparative analyses of *Acacia* gums which have a major arabinogalactan component supports this idea [13]. However, there has been no direct comparison of analyses of arabinogalactans from different tissues of the same species. The arabinogalactans isolated from the gum and from the shells of nuts of *Anacardium occidentale* (cashew) have been analysed independently and there are indeed marked differences in their monosaccharide compositions. The gum arabinogalactan has the following composition: galactose 61%, arabinose 14%, uronic acids 6.3%, mannose 2%, rhamnose 7% and xylose 2% [14, 15], while the arabinogalactan from the shell of the nuts has 71% galactose, 25% arabinose and 4% rhamnose [16]. The direct comparative analyses presented here also show variation in composition which is most marked between the sexual and somatic tissues of *Gladiolus*.

(*b*) *Interaction of* Gladiolus *and* Lilium *extracts with galactose-binding proteins*

Another approach to detecting galactose-containing macromolecules is to screen tissue extracts for

Table 1. Monosaccharide composition of the arabinogalactans from *Gladiolus* and *Lilium* extracts

Monosaccharide	*Gladiolus*					*Lilium*	
	Stigma*	Style*	Style†	Leaf†	Petal†	Stigma†	Style†
Galactose	76.1	85.8	82.4	69.0	64.1	64.1	64.2
Arabinose	20.0	14.2	17.6	25.3	32.6	30.1	31.6
Glucose	4.0	trace	†	†	†	†	†
Rhamnose	0	trace	trace	5.7	3.3	5.8	4.2
Galactose:arabinose ratio	3.8	6.0	4.7	2.7	2.0	2.1	2.0

Results are expressed as percentage (by wt) of each monosaccharide.

*Analysis of the arabinogalactan isolated by tridacnin–Sepharose chromatography [4].

†Analysis of the arabinogalactan isolated by precipitation with β-glucosyl artificial antigen. The arabinogalactan–β-glucosyl artificial antigen complex was hydrolysed directly. The proportion of glucose in the hydrolysate which was derived from the plant extract could not be assessed as the precipitating antigen contributed a high proportion of glucose to the analysis.

their ability to interact with defined galactose-binding proteins by gel diffusion. In this study four galactose-binding proteins were used; three lectins and one myeloma protein. The lectins were tridacnin from *Tridacna maxima* the small giant clam [17], the peanut lectin from *Arachis hypogaea* [18], and RCA-120, the castor bean lectin from *Ricinus communis* [19]. Tridacnin and peanut lectin will bind preferentially to galactosides in the β-configuration [20, 21], while *Ricinus communis* lectin shows a preference for β-galactosides, although α-galactosides are also bound [19]. It is likely that these three lectins have a similar primary requirement for galactosyl residues in the terminal non-reducing position [22, 23]; but may differ in their affinity for sub-terminal glycosyl sequences. The J539 myeloma protein is specific for β 1 → 6 linked galactose oligosaccharides [24] and is apparently not dependent solely on terminal galactosyl residues for a binding interaction [23]: thus a significant content of intra-chain β 1,6-linked galactosyl residues in a molecule with a low proportion of accessible terminal galactose residues would probably fulfil the binding requirements.

The interactions of *Gladiolus* and *Lilium* tissue extracts with the galactose-binding proteins are summarized in Table 2. Tridacnin interacted with all extracts examined. For *Gladiolus*, single precipitin bands were obtained with the stigma surface and style extracts. This is in keeping with the previous finding that each of these extracts have an arabinogalactan with a high galactose content, as a major component. As expected, the other lectins also gave a single precipitin band with both these extracts.

The results for the interaction of the somatic tissue extracts with the galactose-binding lectins were somewhat different: firstly, two precipitin bands were obtained on gel diffusion of tridacnin with both leaf and petal extracts. However, the binding interactions with the peanut and

Table 2. Number of components detected in *Gladiolus* and *Lilium* extracts by gel diffusion against galactose-binding proteins and β-glucosyl artificial antigen

	β-Glucosyl artificial antigen	J539 myeloma proytein	Tridacnin	Peanut lectin	*Ricinus* lectin RCA-120
Gladiolus tissue extract					
Stigma surface	1	2	1	1	1
Style	1	2	1	1	1
Leaf	1	2	2	1	0
Petal	1	2	2	0	0
Lilium tissue extract					
Stigma surface	1	2	2	0	0
Style	1	2	1	0	0
Leaf	1	2	1	0	0
Petal	1	2	1	0	0

The freeze-dried extracts were prepared as described in Experimental. They were reconstituted to the following concentrations in 0.02 M phosphate buffer, pH 7, 0.15 M NaCl: *Gladiolus* stigma and style extracts 10 mg/ml, *Gladiolus* leaf and petal extracts 25 mg/ml; *Lilium* stigma extract 10 mg/ml; *Lilium* style, leaf and petal extracts 25 mg/ml. The β-glucosyl artificial antigen was used at 2 mg/ml; peanut lectin and tridacnin at 10 mg/ml; *Ricinus communis* lectin at 2.5 mg/ml. Unfractionated J539 myeloma ascites fluid was used as the source of the J539 myeloma protein. The lectins were dissolved in 0.02 M phosphate buffer pH 7, 0.15 M NaCl, except tridacnin which was dissolved in 0.15 M NaCl containing 0.01 M $CaCl_2$.

castor bean lectins were not as expected: the leaf extract gave a single band with the peanut lectin but no detectable interaction with the castor bean lectin while the petal extract did not apparently interact with either lectin.

An inference from these experiments is that tridacnin is more effective in binding to the galactose-containing macromolecules in the extracts than the peanut or castor bean lectins. This inference is supported by the results obtained by diffusion of the *Lilium* tissue extracts against the lectins. Thus while each *Lilium* extract interacted with tridacnin, no interaction was detected with either of the other lectins.

Experimentally the myeloma protein was difficult to work with as it bound to the agar medium in which the gel diffusion studies were carried out, presumably via the available galactosyl residues of the agar. For this reason, the diffusion studies were carried out in both agar and gelatine and the bands were not always well resolved. However, notwithstanding these practical difficulties, it was an effective ligand for galactose containing macromolecules in the extracts, with two bands being detected in all cases.

(c) *Fractionation of* Gladiolus *and* Lilium *tissue extracts by tridacnin-affinity chromatography*

The effectiveness of tridacnin in detecting galactose-containing macromolecules in tissue extracts was used as a basis for their fractionation. An affinity column of tridacnin coupled to Sepharose 4B was prepared and used to fractionate the four *Gladiolus* extracts and the *Lilium* stigma surface extract. For each extract a tridacnin-bound and a tridacnin-unbound fraction was obtained.

It is likely that the tridacnin-affinity column separates the arabinogalactans on the basis of their proportion of terminal galactosyl residues: the tridacnin-bound fraction having a high proportion, and the tridacnin-unbound fraction having a low proportion of terminal galactosyl residues. Both groups of arabinogalactans would have the capacity for interaction with and detection by the β-glucosyl artificial antigen: the β-glucosyl antigen–arabinogalactan interaction apparently depends on the presence of a branched 3,6-linked galactan 'core' structure; and covalently associated protein has also been implicated [25]. The proportions of arabinose present do not appear to affect the binding interaction, as complete removal of arabinose from an arabinogalactan did not alter its capacity for binding to the β-glucosyl antigen [3]. Analysis of material precipitated from plant extracts by the β-glucosyl antigen in other studies show that in every case an arabinogalactan is precipitated. The material precipitated may be associated with protein, and there is wide variation in the proportion of galactose to arabinose [7–9].

The capacity of tridacnin-fractionated plant extracts to interact with the β-glucosyl artificial antigen and the four galactosyl-binding proteins was tested (Table 3).

The results show that for *Gladiolus* stigma surface and style extracts the tridacnin-bound fraction contained all the material of the whole extract which was able to interact with the β-glucosyl artificial antigen, that is, no interaction between the tridacnin-unbound fraction and the β-glucosyl antigen was detected. This is as would be expected on the basis of previous work which established that the major stigma surface and style components were arabino 3,6 galactans with high contents of terminal galactosyl residues (16 and 29%, respectively) [4]. The J539 myeloma protein detected a component which binds neither tridacnin nor the β-glucosyl artificial antigen; this is presumably a macromolecular component containing internal 1 → 6-linked galactosyl residues, but having a low content of terminal galactosyl residues.

The arabinogalactans of *Gladiolus* somatic tissues were separated into two major fractions by tridacnin affinity chromatography: both the tridacnin-bound and -unbound fractions contained material which interacted with the β-glucosyl artificial antigen. Of these, the tridacnin-bound fractions of both leaf and petal contained at least two groups of galactose-containing macromolecules, as two distinct bands were obtained on gel diffusion of this fraction against tridacnin or the myeloma protein. However, only one group was detected by the peanut lectin and there was no interaction with the *Ricinus* lectin. These observations presumably reflect variations in detailed binding requirements of the different lectins which was also indicated by the results presented in Table 2.

The tridacnin-unbound fractions of the leaf and petal also contained a group of arabinogalactans. Presumably

Table 3. Components of fractionation *Gladiolus* tissue extracts and fractionation *Lilium* stigma surface extract detected by gel diffusion against galactose-binding proteins and β-glucosyl artificial antigen

	Bound fraction from tridacnin–Sepharose chromatography					Unbound fraction from tridacnin–Sepharose chromatography				
	β-Glucosyl artificial antigen	J539* myeloma protein	Tridacnin	Peanut lectin	*Ricinus* lectin (RCA-120)	β-Glucosyl artificial antigen	J539* myeloma protein	Tridacnin	Peanut lectin	*Ricinus* lectin (RCA-120)
Gladiolus tissue extract										
Stigma surface	1	2	1	N.T.	N.T.	0	1	0	N.T.	N.T.
Style	1	2	1	1	1	0	1	0	0	0
Leaf	1 (weak)	2	2	1	0	1	1	0	0	0
Petal	1	2	2	1	0	1	1	0	0	0
Lilium extract										
Stigma surface	1	2	2	0	0	1	2	0	0	0

Tissue extracts were applied to a tridacnin–Sepharose 4B column, two fractions were collected, the material which did not bind and material which bound was eluted by either calcium-free saline or by 0.1 M lactose. The two fractions were dialysed and freeze-dried and were reconstituted to 10 mg/ml in all cases. The concentrations of the β-glucosyl artificial antigen and the galactose binding proteins are the same as for Table 1

N.T. = Not tested.

* Bands were found close to the well containing the myeloma protein, and were poorly resolved in some cases.

the material present in this fraction has the features of the arabinogalactan required for interaction with the β-glucosyl artificial antigen but insufficient free terminal galactosyl residues for interaction with the lectins. This would be in keeping with the higher average arabinose content of arabinogalactans from *Gladiolus* leaf and petal than from the style and stigma (Table 1). The *Lilium* stigma surface preparation showed a greater resemblance to the *Gladiolus* somatic tissue than sexual tissue extracts in that arabinogalactans were present in both tridacnin-bound and -unbound fractions. Again the *Lilium* stigma arabinogalactan has a higher average arabinose content and a lower terminal galactosyl content (11%) [6] than the *Gladiolus* stigma and style arabinogalactans.

These studies make two points: firstly the structural diversity in arabinogalactans from different tissues of a plant and secondly the utility of lectins to demonstrate this diversity and to isolate the components.

The structural diversity is seen in differences of monosaccharide composition of material precipitated from plant extracts by the β-glucosyl artificial carbohydrate antigen. It is also seen in the number of components precipitated by this technique which could be separated on the basis of their affinity for the lectin tridacnin: a single major component for *Gladiolus* stigma and style and at least two distinct groups of components in the other tissues examined.

The monosaccharide and anomeric specificity requirements of the lectins used in this study are well established: but there is little information on the more detailed requirements, particularly the relative affinity for different glycosyl sequences. This is because of the lack of available oligosaccharides containing different monomers and linkage types which are chemically defined. Similar problems are encountered in defining the detailed specificity requirements of enzymes which hydrolyse heteropolysaccharides [26]; for both the enzymes and the lectins, knowledge of the specificity gives structural information regarding the polymer structure, and vice versa, a knowledge of the polymer structure gives us information about the specificity. To date lectins have not been used extensively for plant polysaccharide characterization [27], but in this study we have shown their potential as tools for both the isolation of arabinogalactans and for providing structural information.

EXPERIMENTAL

Gladiolus gandavensis and *Lilium longiflorum* flowers were purchased locally. Peanut lectin was from IBF, Clichy, France. *Ricinus communis* agglutinin-120 (RCA-120) was from Miles, Elkhart, IN, U.S.A. Tridacnin was purified from *Tridacna maxima* clams according to the method of ref. [17]. Mouse myeloma J539 was a gift from Professor M. Potter (National Institute of Health, Bethesda, MD, U.S.A.) and was passaged in mice by Dr. R. Ceredig (Walter and Eliza Hall Institute, Melbourne, Vic., Australia). The β-glucosyl artificial antigen was a gift from Dr. M. A. Jermyn, Division of Protein Chemistry, C.S.I.R.O., Parkville, Vic., Australia and was prepared by coupling the diazotised 4-aminophenyl β-D-glucopyranoside with phloroglucinol [10].

Preparation of tissue extract from G. gandavensis *and* L. longiflorum. Cut flowers of *Gladiolus* and *Lilium* were maintained in 0.01% 8-hydroxyquinoline citrate and 1% sucrose soln. To avoid contamination of the stigmas with pollen the anthers were removed before they dehisced. Mature pistils were collected 24 hr after flower opening and the stigma surface secretion was washed from the stigmas in Tris·HCl, 0.15 M NaCl, 0.001 M $CaCl_2$, pH 7.4 at 4° by repeated dipping of the stigmas over a period of 15 min. This stigma buffer wash was then dialysed and freeze-dried. After removing the stigmas from the pistils the remaining styles were homogenized in the same buffer at 4°. The style extract was centrifuged at 25 000 *g* for 30 min at 4° and the supernatant dialysed and freeze-dried. Extracts of the leaf and petal tissues were prepared by homogenizing the tissues in EtOH at 80° for 2 min. The EtOH-insoluble material was collected on a sintered glass funnel and washed further with EtOH at 80° until all pigments had been removed. It was then air-dried. This EtOH-insoluble material was then extracted in 0.02 M NaPi buffer, pH 7, 1% NaCl (Pi buffered saline) at 0° for 1 hr, filtered through cheesecloth, centrifuged at 25 000 *g* for 30 min, and the supernatant dialysed and freeze-dried. When required, all extracts were reconstituted in 0.02 M NaPi buffer, pH 7, 0.15 M NaCl to the appropriate concn.

Gel diffusion. Double diffusion was carried out by the microslide method (Gelman Instrument Co.) in 1% agarose containing 0.15 M NaCl and 0.02% (w/v) NaN_3 for 24 hr at 37° in a humidity chamber, then examined for the presence of precipitin lines. The β-glucosyl artificial antigen was used at 2 mg/ml; peanut lectin and tridacnin were used at 10 mg/ml; *Ricinus communis* lectin was used at 2.5 mg/ml. The lectins were dissolved in NaPi buffered saline, pH 7, except tridacnin which was dissolved in 0.15 M NaCl containing 0.01 M $CaCl_2$. Unfractionated mouse myeloma J539 ascites fluid was used as the source of the J539 Immunoglobulin A protein. Gel diffusion with the J539 myeloma protein was performed in both 1% agarose and 5% (w/w) gelatine as the myeloma protein was found to bind to the agar matrix.

Electrophoresis. Cellulose acetate electrophoresis was carried out in Tris–barbital–Na barbital buffer, $I = 0.05$, pH 8.8, using a Beckman Microzone apparatus. After electrophoresis the membrane was stained with β-glucosyl artificial antigen (1 mg/ml in 0.15 M NaCl) for 10 min and destained in 0.15 M NaCl.

Isolation of arabinogalactans by precipitation with β-glucosyl artificial antigen. The arabinogalactans were isolated from the tissue extracts with the β-glucosyl artificial antigen. The complex was collected and washed as described in ref. [7]. The complexes were hydrolysed directly for monosaccharide analysis.

Monosaccharide analysis. The arabinogalactan-β-glucosyl artificial antigen complexes were hydrolysed in 2 ml of 2.5 M TFA at 100°, for 2 hr in a sealed tube under N_2. Insoluble material produced during the hydrolysis of these complexes was removed from the hydrolysate by filtration; it was probably derived from phenols released from the β-glucosyl artificial antigen. The hydrolysate was taken to dryness, reduced with $NaBH_4$ and acetylated [28]. The resulting alditol acetates were separated by GLC on a 1.85 m × 4 mm column of 3% SP2340 on 100/200 Supelcoport.

Tridacnin–Sepharose affinity chromatography. Tridacnin was coupled to Sepharose 4B as previously described [29]. The extracts were dissolved in 0.15 M NaCl and 0.01 M $CaCl_2$, filtered, and loaded directly onto the column (17 × 1 cm) of tridacnin-Sepharose 4B. The conditions of chromatography were as previously described [29], with the exception that the bound material from the leaf and petal was eluted with 0.1 M lactose rather than the calcium-free saline.

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REFERENCES

1. Aspinall, G. O. (1973) in *Biogenesis of Plant Cell Wall Polysaccharides* (Loewus, F., ed.) p. 95. Academic Press, New York.
2. Clarke, A. E., Anderson, R. L. and Stone, B. A. (1979) *Phytochemistry* **18**, 521.
3. Gleeson, P. A. and Clarke, A. E. (1979) *Biochem J.* **181**, 607.
4. Gleeson, P. A. and Clarke, A. E. (1980) *Carbohydr. Res.* (in press).
5. Loewus, S. and Labarca, C. (1973) in *Biogenesis of Plant Cell Wall Polysaccharides*, p. 175. Academic Press, New York.
6. Aspinall, G. O. and Rosell, K. G. (1978) *Phytochemistry* **17**, 919.
7. Jermyn, M. A. and Yeow, M. (1975) *Aust. J. Plant Physiol.* **2**, 501.
8. Anderson, R. L., Clarke, A. E., Jermyn, M. A., Knox, R. B. and Stone, B. A. (1977) *Aust. J. Plant Physiol.* **4**, 143.
9. Clarke, A. E., Gleeson, P. A., Jermyn, M. A., and Knox, R. B. (1978) *Aust. J. Plant Physiol.* **5**, 707.
10. Yariv, J., Rapport, M. M. and Graf, L. (1962) *Biochem. J.* **85**, 383.
11. Jermyn, M. A. (1978) *Aust. J. Plant Physiol.* **5**, 697.
12. Gleeson, P. A. and Jermyn, M. A. (1977) *Proc. Aust. Biochem. Soc.* **10**, 29.
13. Anderson, D. M. W. and Dea, I. C. M. (1969) *Phytochemistry* **8**, 167.
14. Tiomno, F. R. (1946) *Rev. Quim. Ind. (Rio de Janeiro)* **15**, 23.
15. Anderson, D. M. W. and Bell, P. C. (1975) *Analyt. Chim. Acta* **79**, 185.
16. Bose, S. and Soni, P. L. (1973) *Indian J. Chem.* **11**, 996.
17. Baldo, B. A. and Uhlenbruck, G. (1975) *Adv. Exp. Med. Biol.* **64**, 3.
18. Lotan, R., Skutelsky, E., Danon, D. and Sharon, N. (1975) *J. Biol. Chem.* **250**, 8487.
19. Nicolson, G. L. and Blaustein, J. (1972) *Biochim. Biophys. Acta* **266**, 543.
20. Baldo, B. A. and Uhlenbruck, G. (1975) *Carbohydr. Res.* **40**, 143.
21. Pereira, M. E. A., Kabat, E. A., Lotan, R. and Sharon, N. (1976) *Carbohydr. Res.* **51**, 107.
22. Goldstein, I. J. and Hayes, C. E. (1978) *Adv. Carbohydr. Chem. Biochem.* **34**, 128.
23. Baldo, B. A., Neukom, H., Stone, B. A. and Uhlenbruck, G. (1978) *Aust. J. Biol. Sci.* **31**, 149.
24. Glaudemans, C. P. J. (1975) *Adv. Carbohydr. Chem. Biochem.* **31**, 313.
25. Jermyn, M. A. (1980) In preparation.
26. Haug, A. and Larsen, B. (1974) in *Plant Carbohydrate Biochemistry* (Pridham, J. B., ed.) p. 207. Academic Press, New York.
27. Sharon, N. and Lis, H. (1979) *Biochem. Soc. Trans.* **7**, 783.
28. Albersheim, P., Nevins, D. J., English, P. D. and Karr, A. (1967) *Carbohydr. Res.* **5**, 340.
29. Gleeson, P. A., Jermyn, M. A. and Clarke, A. E. (1979) *Analyt. Biochem.* **92**, 41.