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REVIEW

FORM AND FUNCTION OF ARABINOGALACTANS AND ARABINOGALACTAN-PROTEINS

A. E. CLARKE, R. L. ANDERSON and B. A. STONE

School of Botany, University of Melbourne, Parkville, Victoria 3052, Australia and Department of Biochemistry, La Trobe University, Bundoora, Victoria 3083, Australia

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Abstract—The occurrence, isolation, chemistry and physico-chemistry of plant arabino-3,6-galactans and arabino-3,6-galactan-proteins is reviewed. The structural relationships between arabino-3,6-galactans from gymnosperm wood, gum exudates of *Acacia* and other trees, and from plant callus cells and whole tissues are discussed. The nature of these proteoglycans is compared with the arabinose and galactose containing cell wall glycoproteins. Interactions of the arabino-3,6-galactan proteoglycans with carbohydrate binding proteins and with Yariv antigens are described. The utility of these reactions for both cellular and subcellular localization of the proteoglycans is discussed. The possible biological roles of the arabinogalactans and the arabinogalactan-proteins are reviewed.

INTRODUCTION

Macromolecules containing arabinose and galactose have been found in most plant tissues. In some situations they are isolated as polysaccharides free from associated protein; in other situations they occur in covalent association with protein, either as proteoglycans*, in which the protein component carries polysaccharide substituents [1, 2] or as glycoproteins, in which the protein component is substituted by one or more oligosaccharide residues [3, 4].

These two groups represent the ends of a continuous spectrum of macromolecules containing both protein and carbohydrate, but in many instances the groups are quite distinct in their localization and function as well as their chemistry. The arabinose and galactose containing glycoproteins are characteristically associated with cell wall fractions of both higher and lower plants, while the polysaccharides and proteoglycans are widely distributed in plant tissues, and are major components of gum exudates and are also produced by many callus cells in culture.

These polysaccharides and proteoglycans containing arabinose and galactose, the arabinogalactans and the arabinogalactan-proteins are the subject of this review. We have collected information on the structural and physical chemistry of the isolated macromolecules and on aspects of their biology such as their distribution, cellular and sub-cellular localization, biosynthesis and secretion, and here consider these data in relation to the possible function of the arabinogalactans and arabinogalactan-proteins in plant tissues.

* Following Reid and Clamp [2] we have preferred the term 'proteoglycan' for this group of macromolecules, although the term 'mucopolysaccharide' is sometimes used synonymously.

ISOLATION AND ANALYSIS

Arabinogalactan-proteins and polysaccharides containing arabinose and galactose, whose association with protein has not been defined, have been isolated from aqueous extracts of plant material by the classical methods of salt and solvent precipitation, and by ion exchange chromatography in combination with gel filtration [5] and electrophoresis [6]. Recently, affinity chromatography with immobilized galactose-binding proteins such as the lectin from the giant clam *Tridacna maxima* [7] and a galactose-specific mouse IgA myeloma [8] have been used. A different approach to their isolation was initiated by Yariv in 1967 [9] who showed that artificial carbohydrate antigens, prepared by coupling 4-aminophenyl glycosides to diazotized phloroglucinol [10], would precipitate gum arabic and polysaccharides containing arabinose and galactose from soya bean, jack bean and maize flour, if the glycosyl groups of the antigens were in the β-configuration, but not if they were in the α-configuration. This finding has been extended by Jermyn and Yeow [11] to the isolation of high MW polymers containing carbohydrate and protein in the ratio approximately 8:1, from seeds representing all taxonomic groups of higher plants, as well as from other plant tissues [12] and filtrates of cultured callus cells [13]. In each case the protein was remarkable for its high hydroxyproline content and the major monosaccharides were arabinose and galactose.

Choice of methods for following the progress of isolation and assessment of homogeneity of the final preparation of arabinogalactan or arabinogalactan-proteins is difficult because often the preparation apparently contains a continuous spectrum of closely related molecular species as well as other chemically distinct macromolecules. Measurement of parameters such as monosaccharide composition, uronic acid, methoxyl, acetyl and

nitrogen content as well as linkage type and degree of branching, and physical measurements such as intrinsic viscosity, optical rotation and light scattering data have been used to monitor the isolation procedure. The preparation is considered to be homogeneous if variation in these parameters is unimodal [14]. Molecular weight measurements are not always useful for assessing the homogeneity of the preparation as spuriously high values due to aggregation during storage or freeze drying may be obtained [15]. Again, partial hydrolysis of a vulnerable internal linkage during the isolation procedure may give a product which is polydisperse with respect to MW but homogeneous by chemical criteria. Recently a number of distinct components of gums have been partially separated within a narrow MW range, by gel filtration [16, 17].

Isolated arabinogalactans are often associated with protein. This protein may originate from free plant proteins which co-purify with the arabinogalactan during the isolation and be non-covalently bound. Adventitous protein of this nature can usually be removed by physical procedures such as gel chromatography, density gradient centrifugation [18], or treatment with dissociating agents [19]. Often the success of a polysaccharide purification procedure has been assessed by the low nitrogen content of the product, and to this end procedures such as alkali treatment, now known to degrade or eliminate protein [1], have been used. Under these circumstances it is not possible to assess whether the associated protein was originally covalently or non-covalently bound. It seems likely that many 'pure polysaccharides' isolated by such procedures will be shown on re-examination to contain some covalently bound protein or peptide, especially in the light of the recent findings that cellulose [20], glycogen [21], barley β-glucan [22], paramylon [23] and two algal polysaccharides [24, 24a] hitherto regarded as 'pure polysaccharides', contain small amounts of covalently bound protein. The protein associated with cellulose, glycogen and paramylon has been implicated in their biosynthesis and on theoretical grounds it is likely that other polysaccharides will also have an associated protein 'primer'. These findings draw attention to the foresight of Jones and Smith [25] who thirty years ago suggested that the nitrogen of plant gums might be related to their biosynthetic program. Many of the arabinogalactans isolated by solvent precipitation and all of the Yariv antigen precipitated arabinogalactans are associated with a protein component which is not removed by physical procedures [18]. Although a covalent association of the protein is implied, its unequivocal demonstration requires the isolation and characterization of a glycosyl-amino acid or peptide from the linkage region.

So far, only serine and hydroxyproline have been shown to be glycosylated in the arabinose and galactose containing proteoglycans and glycoproteins. Serine (and threonine) are frequent glycosylation sites in animal glycoproteins and proteoglycans; threonine is not commonly involved in carbohydrate–protein linkage points in plants, although recently an unusual xylose–threonine linkage has been described in two plant proteoglycans: the extracellular proteoglycan of the red alga *Porphyridium cruentum* which lacks hydroxyproline [24] and that from maize root cap slime [26]. Hydroxyproline is also a major constituent of animal connective tissue proteins, but is not glycosylated in these situations. Hydroxylysine, which is found in a glycosylated form in certain animal collagens has not yet been found in plant proteoglycans or glycoproteins.

Tentative identification of the amino acid involved in the carbohydrate–protein linkage can be made on the basis of its alkali lability. The glycosyl-serine (or -threonine) glycosidic linkage is usually quite labile in mild alkaline conditions [27], but some stability may be conferred by the proximity of groups such as *N*-terminal serine [271] and 2′ and 3′ hydroxyl groups of arabinofuranosides [276] which may be ionized under the alkaline conditions used. The glycosyl group released by the β-alkoxyl elimination reaction [27] is converted to the corresponding sugar alcohol in the presence of sodium borohydride [28]. Catalytic hydrogenation converts the dehydroalanyl residues arising from glycosylated serines to alanyl residues [29]. Alternatively, alkaline elimination in the presence of sodium sulphite converts glycosidically linked serine (or threonine) to cysteic acid (or 2-aminosulphonylbutyric acid) [30]. These derived amino acids can be separated and quantitated by the conventional techniques for amino acid analysis and this allows estimation of the numbers of glycosylated serine (or threonine) amino acids. Glycosidic linkages to hydroxyproline (and hydroxylysine) are quite stable to alkaline conditions since their structure does not favour the β-alkoxy elimination reaction. This property has been used in isolating hydroxyproline glycosides from glycoproteins [31] and proteoglycans [18, 32].

Various means are available for obtaining the protein and carbohydrate components of the proteoglycans free from each other. However, each of these procedures has limitations as concurrent reactions leading to alteration or elimination of the other component may occur. The protein portion may be removed enzymically [33], by alkaline degradation [18] and by hydrazinolysis [34, 35]. Peptide bonds are cleaved during hydrazinolysis while the carbohydrate chains are resistant to such treatment [36]. However, glycosyl-serine linkages are also vulnerable and serine is lost through β-elimination [271]. In addition, cyclic dimerization of adjacent hydroxyproline residues to give hydroxyproline diketopiperazine may occur [36a]. Under the conditions described by Brown and Kimmins [35, 37] with both hydrazine and hydrazine sulphate present, serine and hydroxyproline diketopiperazine were formed from serine and hydroxyproline hydrazides respectively. They suggest that adjacent hydroxyprolines may not be absolutely necessary for hydroxyproline diketopiperazine formation. After hydrazinolysis of a serine rich glycoprotein, serine was retained in the high MW fraction suggesting that high MW carbohydrate chains attached to serine are retained. The carbohydrate portion may be removed enzymically [11] or by treatment with anhydrous hydrogen fluoride [38], but again whether the degradation is restricted to the carbohydrate components needs to be established for each case.

An idea of the overall organization of the framework of the molecule can be obtained by consideration of physical measurements in conjunction with chemical data derived from conventional methods of partial acid hydrolysis, methylation, periodate oxidation and Smith degradation [6, 39, 40]. Measurement of the relative lability of different glycosidic linkages is often useful in preliminary structural studies, for example arabinofuranosyl and rhamnopyranosyl linkages are

relatively acid labile [41], and are preferentially hydrolysed. Where these monosaccharides are in the terminal position, mild acid hydrolysis yields the free sugar.

The analytical potential of enzymic depolymerization with α-L-arabinofuranosyl, α-L-arabinopyranosyl, L-rhamnopyranosyl, β-D-galactan, β-D-galactosyl and β-D-glycuronyl hydrolases [42–48] has not yet been realized. The success of enzymic techniques in revealing the subtle differences in terminal saccharide sequences of the blood group substances is an encouragement that an analogous approach might yield useful information. Although many of the enzymes are available, their usefulness is limited by the difficulties of rigorously defining their specificity. The natural oligosaccharide substrates are usually difficult to isolate and often the only substrates available are synthetic glycosides. Enzymic hydrolysis also gives some information regarding the configuration of the monosaccharides and the anomeric linkage. Optical rotation studies also give some information, but in many cases the D or L form of the monosaccharides is assumed. Recently a new method based on conversion of the monosaccharide to an identifiable diastereomeric glycoside using an optically active alcohol has been devised [49, 49a].

The data derived from all the available analytical methods allow a partial structure of an isolated arabinogalactan to be written, although often such a structure is only one of a number of possibilities. The question of how much of the total molecule a defined or partially defined sequence represents is also difficult to assess. This problem has recently been discussed in relation to the methylation data for heteroxylans [50].

STRUCTURAL GROUPS OF ARABINOGALACTANS AND ARABINOGALACTAN-PROTEINS

The arabinogalactans can be grouped into three main structural types (Table 1): the arabino-4-galactans classified as Type I by Aspinall [39, 40], the arabino-3,6-galactans classified as Type II and polysaccharides with arabinogalactan side chains. A related but distinct group are the cell wall glycoproteins containing arabinose and galactose [51–53]. In addition to these groups, free arabinans not associated with galactose have been isolated by non-degradative procedures from rape, mustard and soya bean seeds [54–58]. Arabinans are also released from seed pectic complexes by alkaline treatment [54, 59, 60].

The Type I arabinogalactans are usually linear polymers of galactopyranose residues in 1,4-β-linkage, which are more or less substituted by arabinose, possibly as arabinosyl oligosaccharides (Fig. 1). In some preparations rhamnose, xylose or uronic acid residues may also be present. The source and monosaccharide composition of these arabinogalactans are listed in Table 2. No association with protein has been reported for this group.

Table 1. Distribution of plant arabinogalactans

Classification	Sources
Arabino-4-galactans (Aspinall Type 1) [40]	Pectic complexes in seeds, bulbs, leaves, etc. Coniferous compression wood
Arabino-3,6-galactans (Aspinall Type II) [40]	Mosses; coniferous woods; gums, saps and exudates of angiosperms; organs such as seeds, leaves, roots, fruits; media of various tissues in culture
Polysaccharides with arabinogalactan side chains	Gums and pectic complexes

A second group of arabinogalactans, the arabino-3,6-galactans, classified as Type II, are apparently more widespread in plant tissues than are the Type I arabinogalactans. Type II arabinogalactans have been described from representatives of all higher plant families where they have been detected in seeds, leaves, roots, fruit as well as xylem sap (Table 3). These are also frequently found in filtrates and cells of plant tissues in culture (Table 3). The Type II arabinogalactans are also constituents of many exudate gums (Table 4), both of angiosperms, for example the *Acacias* and of gymnosperms, especially in the genus *Larix*. The *Acacia* gums and the larch arabinogalactans are both economically important and represent a significant proportion of the total organic material of the plant of their origin: the larch arabinogalactan may constitute up to 35% of the total heartwood of some species [136, 137] and a single *Acacia* tree may produce more than 2 kg of gum each year [138, 139]. Because of the economic importance of the plant gums, considerable effort has been invested in the exploration of their chemistry and has resulted in the definition of their major structural features.

We will now summarize the structural data for the Type II arabinogalactans. The chemistry of the larchwood and *Acacia* gums have been reviewed in detail elsewhere [6, 138, 139]. The chemistry of the arabino-3,6-galactan-gums other than the *Acacia* gums was reviewed in 1969 by Aspinall [6], and the composition of examples of these gums reported since then are summarized in Table 4. The arabino-3,6-galactans from plant tissues and tissue cultures are typified by the *Lolium multiflorum* arabinogalactan-protein, and the composition of this group is summarized in Table 3.

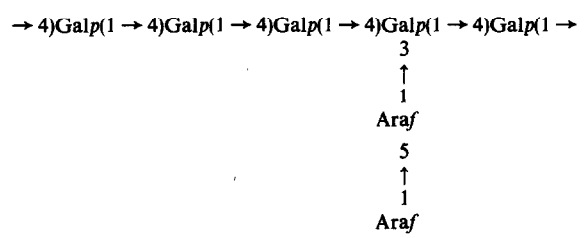

Fig. 1. Proposed structure of the soya bean cotyledon arabino-4-galactan [6].

Table 2. Chemical composition and physical properties of arabino-4-galactans

Source	Monosaccharides (% composition)					Specific rotation	DP	Reference
	D-Gal*p*	L-Ara*f*	Uronic acid	L-Rha*p*	D-Xyl*p*			
Allium cepa (onion) bulb pectic complex	91 (branched)							[61]
Allium sativum (garlic) bulb pectic complex	97.3 (1 → 6 Gal side branches)					+70°		[62]
Beta vulgaris (sugar beet) pectic complex			++			−11° $CHCl_3$		[63]
Camellia sinensis (tea) leaves	66	34				−38.2°	120	[64]
Centrosema plumari seeds	92.5	7				−11° H_2O		[65]
Glycine max (soya bean) flour	53	43		1 (also Glc 3)				[273]
cotyledon	+	+				+20°		[66]
pectic complex	71	29				−17° $CHCl_3$		[67]
Larix laracina (tamarack) compression wood	90 (slightly branched)		GalA					[68, 69]
Lupinus luteus seeds	40	26.6	20	6.6	6.6			[70]
Lupinus albus seeds	+	−	8			52°		[71]
Opuntia ficus (Indian fig)	35.7	37.5		11.5	15.5	−170° −133° $CHCl_3$		[72]
Opuntia dillenii pods	75	25				+266.8		[73]
Phoenix dactylifera (date palm) pollen grains	25.4	45.8	2.2	17.9	8.9	−173° H_2O −140° $CHCl_3$		[74]
Picea rubens (red spruce) compression wood	+		GalA GlcA			+51°	280	[75]
Solanum tuberosum (potato) tuber	94	6				+87° H_2O		[76]
Strychnos nux-vomica seeds	+					−6° $CHCl_3$		[77]
**Symplococcus spicata* leaves	37.5	62.5				+71°		[78]

The arabinogalactan from *Lannea corremandelica* [79] has now been shown to be an arabino-3,6-galactan [80].
* Arabinose is apparently a backbone component as well as a terminal substituent.

Table 3. Chemical composition and physical properties of arabino-3,6-galactans and arabino-3,6-galactan-proteins

Source	Monosaccharides (% composition)								N (protein) %	Specific rotation	10^{-5} MW (DP)	References
	D-Gal*p*	L-Ara*f*	D-GlcA	D-GalA	D-Man*p*	L-Rha*p*	D-Xyl*p*	D-Glc*p*				
SEEDS												
Brassica campestris (turnip rape)	48	46	6						1.01	−49.2°		[81]
Brassica napus (rape)	10	90					tr			−112°		[55]
Coffea arabica (coffee)	71	29								−27°		[82–84]
Glycine max (soya bean)	66	34							very low		3.3	[85, 86]
Triticum aestivum (wheat)	58.4	40.8					0.8		(6.1)	−43.5° (derived galactan −13°)		[87]
	59.2	40.8							(8)	−60° (derived galactan −7°)	0.2	[18, 88]
	79	21							(4.4)		Excluded by Sepharose 4B	[89]

Table 3—*continued*

Source	Monosaccharides (% composition)								N (protein) %	Specific rotation	10^{-5} MW (DP)	References
	D-Gal*p*	L-Ara*f*	D-GlcA	D-GalA	D-Man*p*	L-Rha*p*	D-Xyl*p*	D-Glc*p*				
LEAVES, ROOTS, STEMS, FRUITS, ETC.												
Acer saccharum	51	44				5				−41°		[90]
(maple)	51.0	41.7				7.2						[53]
xylem sap	46.4	38.1				6.7		8.7				[53]
Aegle marmelos	71	12.5	7.0			6.5				−84°		[91–93]
(bael fruit) gum												
Anacardium	85	10	+			5						[94, 95]
occidentale	70.5	25.3				4.3						
(cashew nut) shell												
Asparagus officinalis	53.1	38.4			2.2	6.3						[11]
(asparagus)												
Cannabis sativa												
leaves												
(South Africa) A	28.3	42.5			3.6			25.6	(16.8)	+21	0.165 and 0.83	[96, 97]
B	31.7	30.4	22.8	13.4	4.3			10.8	(25.2)	0°	0.42 and 1.25	
(Thailand) A	37.1	34.5			16.4		2.3	9.7	(19.9)	−12°	0.165 and 0.7	[98]
B	30.2	18.9	27.6	25.7	3.6	10.9	3.7	5.2	(25.0)	+14°	0.28 and 1.10	
Coffea indica	20	80	tr							−103		[99]
(coffee cherries)												
epicarp and												
mesocarp												
Fontinalis												
antipyretica	22	76					tr			+18°	(60)	[100]
(freshwater moss)											(107)	
stem												
Gladiolus												
gandavensis												
style canal	86.7	13.3						tr	(3.0)	−10°	2.4	[101, 101a, 260]
mucilage												
stigma surface	79.4	20.8						tr				[260]
secretion												
Lilium longiflorum	57	26	11			6						[279]
stigmatic												
exudates												
Oryza sativa	45	29				8	7	11	(27.4)			[102, 103]
(rice) bran												
proteoglycan												
Phaseolus												
astropurpureus	53.0	33.2	13.8							+9°		[104]
leaves												
Phaseolus lunatus	50	39.6				8.1			2.2			[11]
(lima bean) seeds												
Phyllostachys												
edulis	50	50					3.4			−26.5°		[105–107]
(bamboo) shoots												
Lycopersicon												
esculentum	59.8	37.9				2.2						[11]
(tomato)												
Vicia faba	43	14	43						(4–7)			[108]
leaves												
Welwitschia mirabilis	14	44	25			5	5			−39.5°		[109]
leaves						(6 fucose)						
SUSPENSION CULTURED CELLS AND MEDIA												
Acer pseudoplatanus	68.6	31.1								−57°		[107, 110]
(sycamore) 2B-1	29.4	31.0		10.4	1.9	1.7	2.1		(19)			[20, 111, 112]
2B-2	27.2	22.4		14.3	0.9	4.2	0.7		(26)			
2B-3	23.2	20.9		7.6	1.4	3.8	0.4		(38)			
Avena sativa	Arabino-3,6-galactan present, but not separated from arabinoxylan.											[113]
(oat) embryo	No arabino-3,6-galactan present in wheat or sugar cane culture filtrates.											
Lolium multiflorum	64	36							(7)		2.2–2.8	[13]
(ryegrass)												
endosperm												
Lycopersicon												
esculentum	74	54							(5)			[51]
(tomato)												
Nicotiana	45	47				8			1	−39.2°	71	[114]
tabacum	44	30	21			5			(6)		6	[115]
(tobacco)												
Phaseolus vulgaris	73.3	26.7								−50°	0.2	[116]
(red kidney bean)												
root												
Vinca rosea	28.7	21.6	22.4		1.2	tr	11.1	15.0	4.7			[117]
(vinca)	Arabino-3,6-galactan present, but not separated from polyuronide and xyloglucan.											

Table 4. Chemical composition and physical properties of arabino-3,6-galactan exudate gums from species other than *Acacia**

Source	Monosaccharides (% composition) D-Gal*p*	L-Ara*f*	D-GlcA (4-*O*-methyl uronic acids)	D-GalA	D-Man*p*	L-Rha*p*	D-Xyl*p*	N (protein) %	Physical properties Specific rotation	Specific viscosity η_{sp} ml/g	10^{-5} MW	References
Anacardium occidentale (cashew)	61 (also glucose 8)	14	6.3		2	7	2	(2.8)	+24.2°	6.3	2.6	[15, 118, 119]
Araucaria bidwillii (bunya bunya pine)	56 65	17 15	18 15			9 5						[120–122] [279]
Azadirachta indica (bead tree)	+++	+++	28		+	tr	tr	6.0 (37.5)	−62°	10.6	5.2	[123, 124]
Bromelia hieronymi fruit exudate	22	41.5	5.5				31					[125]
Citrus limonia (lemon) gum	36	33	7 (16)			5		0.51	+17°	16.6	20	[126]
Combretum hartmannianum	22	43	7.4 (1.5)	6.1	10	4	6	63	−35°	63	6.4	[127]
Commiphora africana gum resin	39.3	52.5				8.2		34.3				[128]
Cussonia spicata (cabbage wood tree) gum	23	58	12			7			−81°		1.25	[129]
Ferula galbaniflua gum resin	56.1	36.3				7.6		3.7				[128]
Lannea humilis A	72.5	13	11.5			3		1.75	+36°	9.6		[130]
B	71	12	12			5		1.81	+43°	8.7		[131]
Lannea coromandelica	69.5	11	17			2.5		1.38	+27°	11.7	2.57	[130, 132]
Lannea schimperi	69.5	10	17			3.5		1.69	+30°	14.4		[130]
Laxopterygium huansango	no analyses given but structure shown											[131, 132]
Odina wodiei (Joel) gum	28.6	50	20			1.4			+55.3°			[133]
Prosopis uliflota (mesquite) gum	++	++	17 (4)			tr			+60°			[134]
Terminalia sericea	22	48	1.6 (7.2)	2.1	7	6	6	0.46 (2.87)	−13°	145	2.1	[135]
Terminalia superba	20	51	5.2 (2.3)	3.1	9	5	4	0.18 (1.12)	+44°	157	40	[135]

* For references up to 1968 see ref. [6].

FINE STRUCTURE OF WELL DEFINED ARABINO-3,6-GALACTANS AND ARABINO-3,6-GALACTAN-PROTEINS

Larch arabinogalactan

Arabino-3,6-galactans are present in the water-soluble hemicellulose fraction which can be extracted from both hardwoods (angiosperms) and softwoods (gymnosperms). They are present in very low concentrations in hardwoods and conifers such as spruce, pine and douglas fir [137], but occur in very high concentrations in the heartwood of all species of larch [135–141].

The polysaccharides are remarkably soluble and will extract directly into water from ground wood. This property distinguishes them from the water-soluble cell wall polysaccharides which can only be removed from wood after delignification. The arabinogalactan is concentrated in the heartwood and is unevenly distributed throughout the other tissues of the trees. In *Larix* both the concentration and distribution of the arabinogalactans vary between species as well as within a single species. The isolated *Larix* arabinogalactan [137] has a framework of 1,3-β-galactopyranose residues (Fig. 2) [140], although variations have been encountered in which some 1,6-linkages are present in the 1,3-galactan main chains. Some of the backbone galactopyranosyl residues are substituted at C(O)6 or C(O)3, and occasionally at both C(O)6 and C(O)3 on the same residue, with single galactopyranosyl residues or disaccharides such as β-Ara*p*-(1 → 3)Ara*f*, β-Ara*f*(1 → 3)Gal*p*, β-Gal*p*(1 → 6)β-Gal*p*. Galactose and arabinose are the major monosaccharides and are present in ratios which vary between 2.6 and 7.8. In some samples glucuronic acid is found in non-reducing terminal positions and may comprise 7% of the sugar residues (*Larix lyallii*, mountain larch) or be absent altogether (*L. occidentalis*, western larch). The larch arabinogalactans generally occur as two components, one high MW with values recorded in the range 37000–100000 (70–95%) and a second component with a lower MW with values in the range 7500–18000 (5–30%) [137, 142, 143, 143a]. However in a recently investigated arabinogalactan sample from *Larix occidentalis* the high molecular weight component accounted for only 20% of the total [143b]. In some *Larix occidentalis* samples the galactose to arabinose ratio is

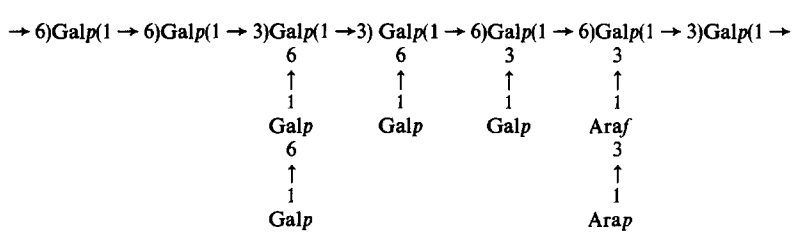

Fig. 2. Partial structure of larch (*Larix laricina*) arabinogalactan [140].

higher in the high MW component than in the low MW component [142, 143a], and the disposition of the arabinose in the two fractions is different [144].

In view of the differing MWs of the two polymers, the finding that they have similar intrinsic viscosities [142] indicates that the lower MW component might be less extensively branched than the high MW component. A recent reappraisal of the structure of larch arabinogalactan using alkaline degradation [145] indicates that arabinose is present not only in the side branches but also in the backbone of the molecule, an observation supported by the earlier finding [143] that mildly acidic conditions, which would cleave only furanosidic linkages, lead to extensive depolymerization of the high MW arabinogalactan (DP 600) to fragments in the range DP 20–48. The alkaline degradation procedure has also led to the conclusion that in addition to single-unit and two-unit branches, a few branches may contain as many as ten residues [145]. Smith degradation of an arabinogalactan from *Larix occidentalis* yielded a 1,3-β-galactan of DP ~12 [143b] indicating that some periodate vulnerable residues are present at regular intervals in the backbone. Whether these correspond to the arabinosyl residues detected in alkaline degradation of another larch arabinogalactan [145] remains to be determined.

There is no evidence for a protein component of larch arabinogalactan. The mild methods used for its extraction and purification might be expected to conserve any protein component, but 0.1% solutions of the arabinogalactan have no absorption above 225 nm [137]. No nitrogen was found in *Larix occidentalis* arabinogalactan [143b]. Larch arabinogalactan is a useful industrial emulsifier, and has been used in lithography and as a non-toxic food additive and tablet binder [146, 147].

Gum exudate arabinogalactans

Plant exudate gums comprise a second major group of Type II arabinogalactans. Their structures have been studied in detail, initially by E. L. Hirst, J. K. N. Jones and F. Smith, and later by Anderson and his colleagues at Edinburgh, as well as by Aspinall's group in Canada, and by Stephen and Churms and coworkers in Capetown. Progress in this field has been reviewed periodically [6, 25, 138, 139].

Gum arabic, a commercially valuable representative of this group, arises as an exudate from the trunks of *Acacia senegal*. Its structure, like that of the larch arabinogalactan, is based on a 1,3-β-galactopyranosyl backbone substituted by 1,6-β-galactopyranosyl side chains. However it differs from both the larch and ryegrass endosperm arabinogalactans (see Figs. 2 and 5) in the variety of glycosyl residues which may substitute the side chains (Fig. 3) [148]. For example, mono- or oligosaccharides involving L-rhamnopyranose, L-arabinofuranose, L-arabinopyranose, D-galactopyranose, D-glucuronic acid and D-galacturonic acid or their 4-*O*-methyl ethers and occasionally D-glucose, have been detected [6, 149] (Table 5).

Table 5. Disaccharides from partial acid hydrolysates of *Acacia* gums [6, 149]

β-D-Gal*p*(1 → 3)-D-Gal
α-D-Gal*p*(1 → 3)-L-Ara
β-D-Gal*p*(1 → 6)-D-Gal
β-L-Ara*f*(1 → 3)-L-Ara
β-L-Ara*p*(1 → 3)-L-Ara
β-L-Ara*f*(1 → 2)-L-Ara
β-D-GlcA(1 → 6)-D-Gal
α-D-GlcA(1 → 4)-D-Gal
4-*O*-Meβ-D-GlcA(1 → 6)-D-Gal
4-*O*-Meα-D-GlcA(1 → 4)-D-Gal

All *Acacia* gums have fundamental structural similarities, but may vary considerably in their overall composition and detailed organization [149, 149a]. They are usually water soluble, but some dissolve only in dilute alkali [138] or after borohydride treatment [17, 149a]. Diversity in MW (0.47×10^5 [150] to 32×10^5 [150a]), N content (0.02% [149] to 1.66% [150a], equivalent to protein content of 0.13% to 10.38%), galactose to arabinose ratio (0.3 [151] to 12 [151]), ratios of 1,6- to 1,3-linked galactosyl residues in the whole molecule and in the galactan backbone [149] and the content (4–16%) and type of uronic acid (galacturonic acid, glucuronic acids and their methoxy derivatives) [152] have all been recorded for different *Acacia* gums. Furthermore, gum samples, even from a single tear, show heterogeneity on ion exchange chromatography [153], electrophoresis [154] and by serological tests [155].

The potential of the fine structural features of the *Acacia* gums as taxonomic markers has been reviewed by Anderson and Dea [149]. In instances where reliable data are available for both the chemical analysis of the gum and the classification of the *Acacia* source, group specific differences can be distinguished. For example, the gums from *Acacia* species in Series 4 and Series 5 of Bentham's classification [156] differ with respect to the

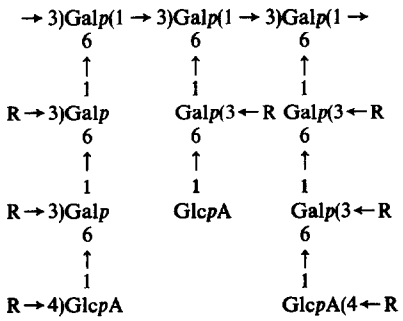

Fig. 3. Partial core structure of *Acacia senegal* gum (gum arabic) where R = Rha*p*(1, Ara*f*(1, Gal*p*(1 → 3) Ara*f*(1, Ara*p*(1 → 3) Ara*f*(1 [148].

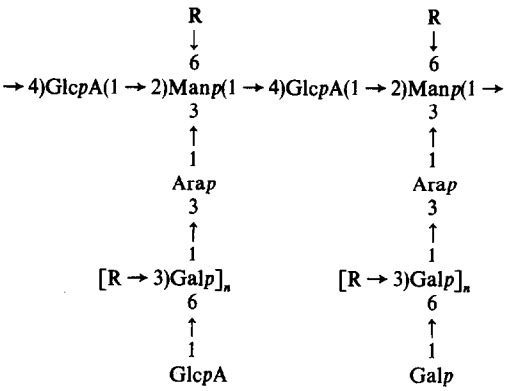

Fig. 4. Proposed structure of *Anogeissus latifolia* gum (gum ghatti) [6], where the majority of sites carry substituents R = Araf-(1, or less frequently, Araf-(1 → 2)-Araf-(1, Araf-(1 → 3)-Araf-(1, or Araf-(1 → 5)-Araf-(1.

ratio of rhamnose to uronic acid, the nature of the terminal oligosaccharides, the length of arabinose containing side chains and the relative proportions of 1,3 and 1,6 backbone linkages [149]. Since Anderson and Dea [149] discussed the use of gum structures as taxonomic markers, gums from further species in Bentham's series 4 and 5 as well as series 1 and 2 have been examined, and some earlier data revised [150–152, 157–159]. The chemotaxonomic aspects of these more recent findings has been reviewed [159a].

Gum production occurs in many other plants apart from those in the genus *Acacia* (Mimosaceae). Most other recorded gum producing plants are angiosperms of the Rosaceae, Meliaceae, Apiaceae and Rutaceae [109]. However a few gymnosperms do produce gums of this type, for example *Araucaria* (Pinaceae) [120, 160], *Encephalartos* (Cycadaceae) [161], and *Welwitschia mirabilis* [109], a remarkable desert plant, which is classified either with the cycads or in an order of its own, the Welwitschiales. Langhammer *et al.* [109] suggest that the structures of the gum exudates in general, progress from the simple galactans and the arabinogalactans of the conifers (e.g. *Larix*) to the more complex gums of the higher angiosperm taxa. The plants producing gum exudates containing arabino-3,6-galactans are listed in Table 4 and references [6, 137, 138, 158].

Although nitrogen content of many gums has been measured and is assumed to be related to their protein content, very few amino acid analyses are available and no demonstration of a carbohydrate–protein linkage has been made. Anderson *et al.* [160] have summarized the reported analyses of a number of gums: serine, threonine and aspartic acid are consistently found in relatively high proportions and hydroxyproline was also qualitatively demonstrated. In addition to these amino acids, *Lannea* gums also have a high proline and leucine content.

Arabino-3,6-galactans as side chains of other macromolecules

Two other groups of macromolecules are related to the arabino-3,6-galactan gums by the structural similarity of sequences of some of the outer chains. For example, gum ghatti (*Anogeissus latifolia*, Combretaceae) [6] has a glucuronomannan backbone to which are attached single arabinofuranosyl units and arabinofuranosyl oligosaccharides through C(O)6 of the mannosyl residues (Fig. 4). The mannopyranosyl residues are themselves substituted through C(O)3 with L-arabinopyranosyl residues and in turn bear chains of 1,6-linked galactopyranosyl residues of variable length, some of which are terminated by 1,6-linked glucuronic acid residues. The galactose chains are also substituted through C(O)3 with L-arabinofuranose and arabinofuranose oligosaccharides. Similar side chains with or without an L-arabinopyranose 'link' are found in other gums and beech tension wood [6].

Components of pectic complexes with galacturonorhamnan backbones to which arabinogalactan side chains are attached have been described from *Acer* callus cell walls [60, 161a, 162], *Panax ginseng* [163, 164], *Zostera marina* [164–166] and *Hibiscus ficulneus* [167]. The side chain may be attached either to the rhamnose residues, as in the *Panax* pectin, or to the galacturonic acid residues, as in the *Zostera* pectin.

Arabinogalactans and arabinogalactan-proteins related to the Lolium multiflorum *arabinogalactan-protein*

The third group of arabinogalactan proteins found in plant tissues are typified by the ryegrass, *Lolium multiflorum*, arabinogalactan-protein which is found both in the cells and the media of suspension cultured *Lolium* endosperm [13]. The *Lolium* arabinogalactan-protein has a MW of 200000–300000 and contains approximately 95% carbohydrate associated with 5% protein. The carbohydrate component consists almost entirely of galactose and arabinose in the ratio 1:1.7. No uronic acids have been detected. The isolated material has a backbone of 1,3-β-galactopyranosyl residues, and side branches of galactopyranosyl residues, or oligosaccharides linked through C(O)6 to the backbone galactose residues. The side branch galactose residues are in turn substituted by 1,3-linked arabinofuranosyl residues and the terminal galactose residues may be substituted by either 1,6- or 1,3-linked arabinofuranose residues, or both. The representation in Fig. 5 is only one of several possible interpretations of the analytical data. Individual preparations show some heterogeneity with respect both to their behaviour on anion exchange resins and towards galactan-binding proteins [Taylor, I., Andrew, I. G. and Stone, B. A., unpublished data].

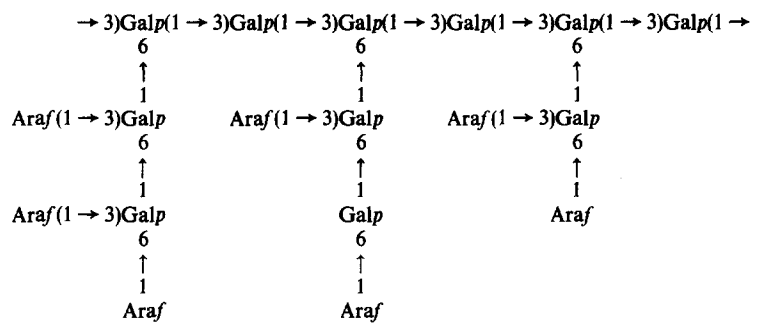

Fig. 5. Tentative structure of ryegrass (*Lolium multiflorum*) endosperm arabinogalactan-protein [13].

The physical and chemical composition of the other related water-soluble arabinogalactans from leaves, roots, stems and tissue culture media are summarized in Table 3. They resemble the gums in their diversity of MW (25000–330000), their polydisperse nature, and the variety of minor monosaccharide components. Small and variable amounts of mannose, rhamnose, xylose and uronic acids and occasionally glucose have been reported in some preparations. The arabinose is invariably terminal and is sometimes in the pyranose form (*Fontinalis antipyretica* [100], *Nicotiana tabacum* [114]). All preparations investigated show a low positive or negative optical rotation probably reflecting the preponderance of the β-D- and α-L-configurations of the galactose and arabinose residues, respectively. The protein content in most cases has been estimated from the total N analysis. Since amino sugars are usually present only in very small amounts, the N is likely to be derived from protein. Values vary considerably from one sample to another up to an extent equivalent to *ca* 7% protein.

A large number of representatives of this group of arabino-3,6-galactans has been isolated by precipitation of plant extracts with the β-glycosyl artifical antigen (Yariv antigen) [11]. Because of their ability to bind these β-glycosyl dyes, these arabinogalactan-proteins have been referred to as β-lectins. Normal hapten inhibition tests with β-glycosides are not generally effective but a galactose containing flavonol glycoside has been shown to inhibit the reaction between β-lectins and Yariv antigens [168]. Analysis of the stereochemical requirements for binding show that the Yariv artificial antigen must bear a glycopyranose residue with the β-D- or α-L-configuration at C(O)1 and the D-*gluco* configuration at C(O)2. A further requirement is the 1:4 orientation of the azo and glycosyloxy groups to the phenyl ring. The basis of the reaction is not completely understood. Whether the binding involves the carbohydrate or the protein component of the arabinogalactan-protein, or perhaps both components, has not been established. However, the reaction has been extremely useful, not only for the isolation of the arabinogalactan-proteins but also for their histochemical localization in plant tissues [13, 174] and at protoplast surfaces [175, 176].

Amino acid analyses published for several arabinogalactan-proteins isolated by precipitation with the β-glycosyl Yariv antigens show a characteristically high alanine, hydroxyproline and serine content [11, 177]. So far, no polypeptides from proteoglycans have been isolated in an intact form for amino acid sequence studies. Treatment of glycoproteins with anhydrous hydrogen fluoride [38], or hydrogen fluoride in pyridine (Roberts, K., pers. commun.), is reported to cleave all *O*-glycosidic linkages of neutral sugars without altering the peptide bonds. This procedure might be expected to yield the protein fragment of the proteoglycans in a form suitable for sequence analysis. Jermyn [177] obtained a 'core peptide' from a number of arabinogalactan-proteins by enzymic degradation. These 'cores' showed remarkably constant amino acid compositions, suggesting a protein structure which is apparently conserved in the evolution of plants. In most analyses for amino acids, hexosamines have been reported in amounts of up to 2%, and the presence of a basic amino acid which behaves chromatographically as ornithine has been noted in potato lectin [189, 276], the 'all β-lectins' of a number of plants [11], the *Lolium* arabinogalactan-protein [13] and a water-soluble proteoglycan from *Vicia faba* [108]. The structural significance of these components is not known.

The linkage between the polysaccharide and protein in this group has so far received little attention, although hydroxyproline has been implicated as a linkage amino acid because of its characteristic presence in these arabinogalactan-proteins. The stability of the galactan portion of the wheat endosperm arabinogalactan-peptides to alkaline conditions (5M NaOH, 100°, 24 hr) and the enrichment of the resulting product with respect to hydroxyproline led to the suggestion [18] that galactosylhydroxyproline glycosidic linkages were involved. This was subsequently confirmed by isolation and identification of 4-*O*-β-D-galactopyranosyloxy-L-proline following chemical and enzymic degradation of the sample [32]. Recently alkali-labile D-galactosyl-*O*-serine linkages were demonstrated in the proteoglycan isolated from *Cannabis sativa* leaves [96, 98] and these presumably link the main arabinogalactan chains to the protein. In the arabinogalactan-proteins isolated from *Cannabis* leaves from South Africa [96] but not in those from Thailand [98], a second glycosyl-amino acid linkage, which is alkali-stable and believed to be glycosylhydroxyproline has been detected [97]. Thus, as in a number of animal and plant glycoproteins [3, 4], more than one linkage type is encountered in the same molecule. The linkage types reported from arabinogalactan-proteins are collected in Table 6.

The structures of arabino-3,6-galactans from larch wood, gum arabic and ryegrass endosperm illustrate both the basic similarity of the overall architecture as well as the diversity of structural detail. Although all

Table 6. Proposed carbohydrate–protein linkages in plant proteoglycans and glycoproteins containing arabinose and galactose

Linkage Type	Proteoglycan Source	Anomeric configuration	Glycoprotein Source	Anomeric configuration
Galactopyranosyl-4-*O*-hydroxyproline	Wheat endosperm arabinogalactanpeptide [18, 32] *Acer* culture filtrate [230]	β [18, 32]	*Chlamydomonas* cell wall protein [184, 53]	
Galactopyranosyl-*O*-serine	*Cannabis sativa* leaves [96, 98]		Cell wall glycoprotein (extensin) [171, 172, 53, 271]	
			Potato lectin [276]	α [276]
Arabinofuranosyl-4-*O*-hydroxyproline	Rice bran proteoglycan [103]	α [103]	Cell wall glycoprotein (extensin) [169, 53, 272]	α [169]
			Potato lectin [188, 276]	β [276]

are basically built in a branched β-galactan framework, its organization into a comb-like structure or a branch-on-branch structure, the frequency of branching and the types of inter-residue linkages in the main chains are variables. So too are the qualitative and quantitative range of terminal and near-terminal sugars and the configuration and position of the glycosidic linkages by which they are joined to the backbone. Altogether these possibilities allow the generation of a wide range of subtly different molecules.

Glycoproteins containing galactose and arabinose

The arabinogalactan-protein (proteoglycan) molecules discussed in this survey can be distinguished from the arabinose- and galactose-containing glycoproteins found in plant cell walls, and sometimes referred to as 'extensin' [20, 31, 169–172]. The name 'extensin' was adopted to convey the involvement of this glycoprotein in the control of elongation growth. In these polymers, arabinose oligosaccharides are glycosidically linked to hydroxyproline and the galactose residues to serine as shown in Fig. 6 and Table 6. The glycoproteins appear to be intimately associated with the water-insoluble cell wall polysaccharides, especially cellulose [173], but have not yet been isolated in their native form. They are no doubt functionally distinct from the water-soluble arabinogalactan-proteins. Apparently related glycoproteins have been reported in the cell walls of normal and virus-infected leaves of *Phaseolus vulgaris* [178–181]. Apart from arabinose and galactose, xylose and glucose are also found in the oligosaccharide side chains, and the carbohydrate composition of these glycoproteins seems to be related to the wounding and the progress of the viral infection. Details of the carbohydrate–protein linkage are not known, both hydroxyproline and serine are apparently involved and both are linked to the three major monosaccharides, galactose, glucose and arabinose. Some hydroxyproline residues are apparently linked to arabinose only. Selvendran and coworkers [182, 183] have demonstrated the presence of similar glycoproteins in cell wall preparations of *Phaseolus coccineus*; two glycoprotein fractions characterized by their relatively high and low hydroxyproline contents

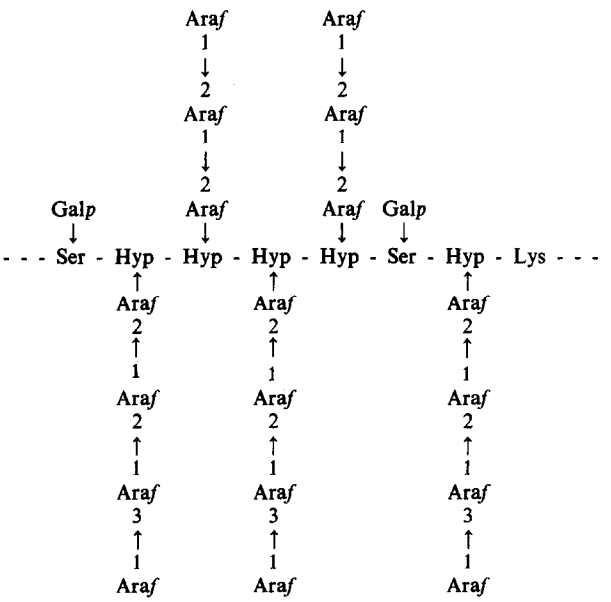

Fig. 6. Possible structure of cell wall glycoprotein segment based on studies by Lamport *et al.* [271] and Akiyama and Kato [170].

were separated and both contained glucose, xylose, rhamnose and uronic acid in addition to galactose and arabinose.

Glycoproteins rich in hydroxyproline, arabinose and galactose have also been found to be a major cell wall component of the green alga *Chlamydomonas reinhardii* and are present in wall preparations of other green algae [184, 185, 185a]. The glycoproteins from *C. reinhardii* can be extracted and reassembled *in vitro* to give a crystalline wall lattice [186, 187].

The potato tuber galactose-binding lectin is a glycoprotein with a structure resembling the cell wall glycoprotein in its arabinose–hydroxyproline linkage, but is relatively rich in arabinose and is water soluble [188]. Recently, Muray and Northcote [189] re-examined this glycoprotein and compared it with the insoluble cell wall glycoprotein from potato callus cells. Their results suggest that both the soluble (lectin) and the insoluble (wall) glycoprotein contain hydroxyproline residues glycosylated by short chain arabinose oligosaccharides. The finding that no large oligosaccharide fragments were attached to hydroxyproline in the lectin, allows its classification as a glycoprotein rather than a proteoglycan. Simultaneously Allen, Desai, Neuberger and Creeth [276] published a comprehensive account of the chemistry and physico-chemistry of potato lectin. They find that all the arabinofuranosyl residues, including those directly linked to the hydroxyproline residues, are β-linked. The ratio of arabinofuranose to hydroxyproline was 3:4:1 in agreement with the estimate [189] of 3 or 4 arabinose residues in each chain. In addition 9 of the 10 serine residues in the polypeptide are substituted with single α-galactosyl residues. Other water-soluble arabinose- and galactose-containing glycoproteins have been described [190, 230, 266, 272].

IMMUNOCHEMICAL AND LECTIN PROBE APPROACHES TO ARABINOGALACTAN-PROTEINS

Characterization and localization

The use of affinity columns of galactose-binding proteins in the isolation of arabinogalactans has been mentioned. A number of β-galactose binding lectins are commercially available. For example, those from the castor bean (*Ricinus communis*) [191, 192], peanut (*Arachis hypogea*) [193] and *Abrus precatorius* [194]. Other β-galactose binding lectins such as that from the giant clam (*Tridacna maxima*) [195, 196] can be prepared and a number of mouse IgA myeloma proteins are available [197–199] whose specificity includes 1,6-β-galactopyranosyl residues [200]. These all have a requirement for galactose in the ligand, but have differing affinities for galactose oligosaccharides and substituted galactose residues. The variation in their detailed binding requirements is seen in the patterns of precipitation of a particular arabinogalactan with a number of galactose-binding proteins, some being very effective and others being much weaker precipitants of the arabinogalactan [201]. Thus the myeloma protein from J539 ascites cells binds both the *Lolium* arabinogalactan-protein and the wheat endosperm arabinogalactan-peptide and the derived galactan-peptides, but the tridacnin shows affinity only for the galactan-peptides [201].

Lectins specific for other terminal substituents of the arabinogalactan-proteins, such as arabinose, rhamnose and the uronic acids would be extremely useful for their isolation and characterization. However, all the screening tests for lectins to date have been based on haemagglutination reactions which will only detect lectins specific for the sugars of the erythrocyte cell membrane. Since arabinose, rhamnose and the uronic acids are not constituent sugars of this membrane, lectins specific for these sugars have not been found.

Some arabinogalactan-proteins and gums will react with antisera raised to the Type II and Type XIV pneumococcal polysaccharides [139, 155, 202–205]. The major antigenic determinant of the Type II polysaccharide is its terminal α-glucuronyl unit, and the Type XIV polysaccharide has a terminal β-galactosyl determinant [206, 207]. The cross reactivity of arabinogalactan-proteins and gums has been shown by hapten inhibition tests to depend upon the presence of the same glucuronic acid or galactose determinants in the plant material.

The arabinogalactan-proteins themselves are weakly antigenic [208, 209]. The antigenic nature of connective tissue proteoglycans has been attributed to the polypeptide chains, and no contribution to the antigenicity of the molecule from the polysaccharide component could be detected [210], but the relative contribution of the individual components of the arabinogalactan-proteins is not known.

PHYSICOCHEMICAL PROPERTIES

Physicochemical studies have largely been restricted to the gum arabinogalactan-proteins and the larch arabinogalactans. The gums are secreted as sticky fluid drops which dry to a glassy solid. The nature of the solid gum changes visibly during storage, perhaps due to the slow action of enzymes secreted with the gum. The gums in solution are usually polydisperse with respect to MW, and are likely to be heterogeneous with respect to peripheral saccharide residues so that physical measurements will refer to an average molecular species. Some idea of the shape these molecules adopt in solution is given by measurements of viscosity and light scattering. The low viscosity of larch arabinogalactan in aqueous solutions (η_{sp} 4.80 ml/g) [211] is consistent with a spherical shape perhaps arising from a molecule with a branch-on-branch structure as in amylopectin. Analysis of the viscosity data for gum arabic (*A. senegal* gum) by the Mark–Houwink equation [212] and by light scattering [211, 213] suggests they are highly compact molecules with a radius of gyration of 100 Å. This is in agreement with the high degree of branching indicated by chemical analysis [211, 213]. Confirmation of their globular rather than extended form is given by measurements of the end-to-end distance of the molecules which differ significantly from the calculated values for an extended linear molecule in solution. The resulting spherical particles may in turn be organized into rod-shaped aggregates [214]. The overall shape and behaviour in solution of the arabinogalactan proteoglycans will be influenced by the extent of substitution of the proteins by polysaccharide chains and the shape and flexibility of the individual substituent chains. The substituent chain characteristics will in turn be determined by such parameters as the linkage type, degree of branching and content of charged uronic acid residues which interact strongly with aqueous solvents [211].

Fractions of *A. senegal* gum showed an increasing viscosity (η_{sp} 11–20 ml/g) with increasing MW ($1–20 \times 10^5$) and protein content. The viscosity was unaltered in the presence of 1 M NaCl, suggesting that the protein was an integral part of the molecule, although a covalent protein–carbohydrate linkage was not established. Neither the uronic acid content nor the methoxyl group content apparently contributed to the observed viscosity differences as the analytical data showed these parameters to be essentially constant in the individual fractions [212, 215, 216].

A relationship between degree of branching and viscosity of other gums is apparent: thus the *Lannea humilis* gum [130] is highly branched and has a low viscosity (η_{sp} 9.6 ml/g; MW 3.1×10^5; protein 1.75%) while the *Combretum hartmannianum* gum [127] contains long, sparsely branched chains of 1,6-linked galactose residues which can assume an extended molecular shape compatible with its higher viscosity (η_{sp} 63 ml/g; MW 6.4×10^5; protein 4%) and the gum from *Terminalia superba* [131] is extremely viscous (η_{sp} 157 ml/g; MW 40×10^5; protein 1.12%). Although the degree of branching of this gum has not been determined, it might be expected to be essentially linear. The uronic acid content of these gums did not apparently account for their different viscosities, thus the *Lannea* gum contained 13%, the *Combretum* gum 15% and the *Terminalia* gum 11% uronic acid.

It is of interest that preparations of larch arabinogalactan, virtually free of uronic acid, give solutions of extremely low viscosity even at high concentrations [137]. Anderson [217] has suggested that the relative hydrophilic character of a gum may be determined by the rhamnose:uronic acid ratio, the rhamnose giving the hydrophobic and the uronic acid giving the hydrophilic characteristics. The sharp titration curves of the gums indicate the generalized terminal location of the uronic acid residues [218].

The shape of the individual galactan chains is not known, but computer model building [224] has shown that individual galactan chains will form open helices as do their 1,3-β-glucan and 1,3-β-xylan counterparts. X-ray analysis has shown that the individual helical molecules of some 1,3-β-glucans and xylans in the solid state may associate or be induced to form a triple-stranded rope which is stabilized by triads of hydrogen bonds at C(O)2 on each strand [219, 220]. It is relevant that C(O)6 substituted 1,3-β-glucans may also be induced to aggregate in this way [221]. This is possible since the C(O)6 substituents are on the outside of the triple helix and hence substitution does not interfere with chain packing. Computer calculations [277] show that it is theoretically possible for 1,3-β-galactan molecules to form triple helices and, by analogy with the substituted 1,3-β-glucans, it would also be possible for 1,3-β-glactans substituted through C(O)6 to organize into a triple helix which would have the form of a 'hairy rope'.

A classical property of gums is the adhesive nature of their solutions. The characteristics of an ideal adhesive base have been described for synthetic polymers [223]: it should be a soluble polymer of a high average MW and narrow MW distribution, with a flexible, deformable backbone. Features such as degree and nature of substituents, variation of sequence of monomeric backbone units, cross linking of macromolecular constituents, are varied to a degree which optimizes the balance between loss of flexibility with a resulting brittle adhesive film and a softer 'tacky adhesive with reduced cohesive strength. The arabinogalactan-proteins could, on these criteria, be regarded as approaching the specification of an ideal adhesive base.

The backbone 1,3-galactan chain provides a flexible linear high MW polymer which may be more or less substituted by galactose:arabinose oligosaccharide chains of variable length. The flexibility of the molecule may be enhanced by introducing other linkages, particularly 1,6-linkages which have three rather than two torsion angles [224]. Charge variability is controlled by the presence or absence of uronic acids in these substituent chains. The linking of arabinogalactan units to protein chains increases the effective molecular weight of the polymer and further orders the molecule.

The molecules in solution aggregate and the highly branched structure with regions of regular carbohydrate sequence provide ideal situations for interactions with other macromolecules. Coacervates between these molecules and proteins in solution might be expected to produce a gel [138] and interaction with low MW carbohydrates might be expected to increase the 'tackiness' of the gum [223]. All these properties may be important to the function of the molecules in the plant of their origin.

BIOSYNTHESIS

The little information available on the biosynthesis of the arabinogalactan-proteins can be considered at the following levels: synthesis of the protein component, the monosaccharide units and the protein–carbohydrate linkages; elongation of carbohydrate chains; intracellular transport of the preformed or growing polymer; secretion and extracellular transport of the product; and overall control of the biosynthesis.

There are no direct studies of arabinogalactan-protein biosynthesis, but by analogy with the synthesis of the cell wall protein and animal glycoproteins, it might be expected that the protein component be synthesized on the rough (ribosomal) endoplasmic reticulum with proline being incorporated into the nascent protein and enzymically hydroxylated as a post-translational event [225, 226]. The plant peptidyl-proline hydroxylase is similar to the animal tissue protocollagen proline hydroxylase [227], in its requirement for ferrous iron, ascorbic acid and an α-keto acid [225, 228] as well as molecular oxygen to provide the oxygen atom of the hydroxyl group [229].

Most biosynthetic studies have relied on specifically labelling the hydroxyproline by supplying labelled proline and following its progressive incorporation into a particular cell fraction. Synthesis of hydroxyproline containing macromolecules associated with both the soluble fraction and the cell walls of *Acer* suspension cultures have been studied in this way [230]. The mechanism of formation of the protein–carbohydrate linkage is not established, but the enzymes concerned with synthesis of both molecular species are associated with a particulate fraction which could originate either from the endoplasmic reticulum or Golgi apparatus [230]. Enzymic glycosylation of hydroxyproline residues with arabinose, and serine residues with galactose, during biosynthesis of the cell wall glycoprotein has been studied [231] and it has been suggested that the sequence

ser-hyp-hyp-hyp-hyp could be the recognition signal for both glycosylases [232]. The glycosyl transferases involved in the glycosylation of hydroxyproline with galactose during biosynthesis of arabinogalactan-proteins have not been described. An assumption of all these studies is that the hydroxyproline is only found in the cell wall glycoprotein or in the arabinogalactan-protein; this may not however be the case. Also arabinogalactan-proteins devoid of hydroxyproline [98] are known and would not be detected by the hydroxyproline incorporation technique. It is likely that the proportion of hydroxyproline incorporated into a particular macromolecule will vary both with the cell type and with the physiological state of the cell, as well as the nature of the macromolecule. The hydroxyproline of the soluble proteoglycan does not appear to be the precursor of that in the cell wall fraction [189, 230, 232].

The intermediate hydroxyproline-containing backbones of both the cell wall glycoprotein 'extensin' and the proteoglycan in *Acer* are associated with the same membrane fraction [230]. This observation suggests that in order to differentiate between the two groups of macromolecules in synthetic studies, it will be necessary to monitor some characteristic specific to each of the macromolecules produced. For example, the gal-hyp and ara-hyp linkage groups might be useful markers in some cases. In higher plants, the incorporation of glucosamine into polymers, which in view of their water solubility might correspond to proteoglycans, has been followed by specific labelling with [1-^{14}C]-glucosamine. The label was recovered as glucosamine from radioactive peptide released by proteolysis [233].

The metabolic pathways for formation of the monosaccharides which are incorporated into the carbohydrate chains, galactose, arabinose, rhamnose and the uronic acids are established [234] and the mechanism for methyl ester formation is known [162]. As already noted arabinose is encountered in both the pyranose and furanose form sometimes in the same molecule but the mechanism of the pyranose–furanose ring contraction is not known. Many of the epimerizations, oxidations, decarboxylations and oxido-reductions involved in monosaccharide interconversions occur with nucleoside diphosphate sugars as substrates [235]. These include ring formation of apiose and galactofuranose from pyranose precursors [234], so it is likely that ring contraction will occur at this level, although other possibilities have been considered [234]. Evidence for some control of biosynthesis of cell wall components at the level of enzymic modification of nucleotide sugars has recently been obtained [236]. Apart from this, no information is available regarding the mechanism or the control of addition of monosaccharides to the polysaccharide chain. Elongation of the carbohydrate chains could occur either by sequential addition of monosaccharides to the glycosylated backbone, in which case the protein may be considered as a primer presenting multiple sites for initiation of glycosylation and chain growth [1, 3, 4] or possibly the monosaccharides may be assembled on some kind of intermediate and added in a block. Although nothing is known of the glycosyl transferases or the nucleotide donors, the evidence from complex carbohydrate biosynthesis in animal and microbial systems, suggests that glycosyl transferases specific with regard to the sugar transferred, the acceptor, and the position and anomeric configuration of the linkage formed, and behaving in accordance with the one enzyme-one linkage concept [237] will be involved [238]. From this we might expect that each terminal disaccharide unit (Table 5) formed in the arabinogalactan-proteins would correspond to a glycosyl transferase specific for the transfer of the non-reducing terminal unit of the disaccharide. In this case, differences in the terminal sequences of side chains (see Table 5) would be a reflection of the genetically controlled expression of a different complement of glycosyl transferases and possibly nucleotide sugars. Thus glycosyl transferases as well as the different disaccharides of the terminal sequences of the arabinogalactan-proteins may be useful taxonomic markers. However, the analytical data are not yet available to state even whether identical arabinogalactan-proteins are produced from different sites of the same plant, or from the same tissue under different physiological conditions, let alone whether the glycosyl transferases present are of taxonomic significance. Anderson and Dea [149a] have compared compositional analyses of gums from trees of single species of *Acacia* and found that the variation between nodules from one tree is considerably less than between samples from different trees. They conclude that in the genus *Acacia* the gum exudates are characteristic of a particular tree of that species. Samples of gum from *Acacia senegal* produced from upper branches by tapping or natural exudation or from trees infested with wood-boring beetles do not vary significantly but the gum exuded from the main stems of trees had a lower rhamnose content and a lower proportion of free uronic acid residues than the gum from the upper branches [239]. A comparison of the arabinogalactan from the gum and shells of the nuts of *Anacardium* (Tables 3 and 4) shows that there are differences in their monosaccharide composition. Rosik [240] has also demonstrated structural changes in apricot gum after gummosis induced chemically or by infection with *Cytospora cincta*. These data suggest different expressions of biosynthetic capacity in different parts of the same plant, but further detailed analyses are required to clarify the issue. The addition of glycosyl residues may occur directly to the growing carbohydrate component of the proteoglycan or from some kind of intermediate from which the oligosaccharide chains may be transferred in a block. This type of addition, in which oligosaccharides are pre-assembled on a lipid intermediate, is known to occur during biosynthesis of many animal glycoproteins [238] and also occurs during yeast mannan biosynthesis [241] and glycoprotein synthesis in *Phaseolus vulgaris* [242].

The possibility of block addition of a preformed fragment during arabinogalactan biosynthesis is suggested by the finding that certain native *Acacia* gums [16, 243–245] appear to contain a series of discrete components with MWs which are multiples of 6000. Similar polymer homologues can be obtained from polydisperse *Acacia* gums [16, 243–245] and *Larix occidentalis* arabinogalactan [144a] following Smith degradation. The analyses indicate that they arise by attack at periodate vulnerable sugar residues distributed regularly along the otherwise resistant 1 →3 linked galactose chains [245]. It is also interesting that the native larch [137] and *Cannabis* arabinogalactans [96] and *Acacia mabellae* gum [245a] occur naturally in a high and low MW form. Whatever the method of chain elongation, there must be some means of termination of chain growth and a signal for secretion of the completed molecule. It is presumed

that glycosylation proceeds as the molecules pass from the endoplasmic reticulum to the Golgi apparatus after which they are transported to the plasma membrane and secreted. Although the precise mode of secretion across the plasma membrane is unknown, the process of secretion of polysaccharides and soluble hydroxyproline containing protein from sycamore suspension cells has been shown to be calcium-ion dependent [246].

There is no information available regarding turnover of arabinogalactan-proteins in plant tissues, although extracts of seeds, leaves and roots of a variety of plants have yielded preparations which hydrolyse β-galactans [247], β-D-galactosides [248], α-D-galactosides [248, 249], β-D-glucuronides [250–253], β-L-rhamnosides [254, 255], α-L-arabinofuranosides [42] and α-L-arabinopyranosides [256]. In no case have the extracts been tested for their ability to cleave specific linkages in arabino-3,6-galactans. Hydrolases for β-L-arabinofuranosidic, α-L-rhamnopyranosidic, D-galacturonosidic, 4-*O*-Me-D-glucurono and 4-*O*-Me-D-galacturonosidic linkages encountered in some arabino-3,6-galactans have not been described. Gums are known to be produced by stress conditions such as heat, drought and wounding, but the question of whether the arabinogalactan-proteins are continuously synthesized and degraded or whether they are synthesized only in response to a physiological need is unresolved.

LOCALIZATION

Although arabinogalactans and arabinogalactan-proteins have been extracted from tissues of flowering plants of every taxonomic group, investigations of their localization within the tissue have been hampered by their extreme solubility and by the lack of specificity in the conventional cytochemical methods for detection of polysaccharides. These difficulties have been partly circumvented in studies of the localization of larch arabinogalactan which is present in huge quantities in the tree and can comprise up to one third of the total wood weight [136, 137]. It is concentrated in the heartwood where it is seen as an intracellular accumulation of amorphous material in the lumen of tracheids [136]. Identification of the arabinogalactan in larch wood was based on its water solubility and polyethylene glycol insolubility and confirmed by hydrolysis of the amorphous material removed from individual tracheids by micromanipulation [141]. It has also been observed as masses under the bark of trees accumulating in response to injury [146]. It is amazing that while we have a wealth of chemical data available for the gums, there is so little information regarding the tissues and cell types involved in their formation. The gums are generally exuded from cracks and fissures in the bark of trees [282], but may also occur at abscission layers and even on the surface of whole peach fruit (J. Raff, pers. commun.). There are indications that where they occur as a mixed oleogum resin, they are secreted from the resin canals [128]. For example, myrrh is a typical oleogum resin composed of carbohydrate, protein and terpenoid polymers and is secreted from the resin canals of *Commiphora myrrha* to form nodules of a red oily resin mixed with white streaks of gum on the bark [257]. The gum exudate of a cycad has also been found in the radial canals [258].

Anatomical studies with *Acacia senegal* [282] have shown that the spontaneously exuded gum comes from 'gum cysts' which develop in one or two tangential rows in the axial parenchyma strands of the phloem adjacent to the cambial zone. The appearance of the gum cysts is preceded by profuse development of parenchymatous tissues. The small cysts are surrounded by parenchyma (secretory?) cells which eventually disintegrate, thereby enlarging the cysts. No ultrastructural information is available. Recently a histochemical and ultrastructural study of the bark and wood of *Commiphora mukul* the source of the commercial gum resin 'guggul' has been reported [278]. This product is secreted into gum resin ducts found in both the primary and secondary phloem. The secretory cells lining the ducts were apparently metabolically active and had thin cell walls with fibrillar material arranged in a loose mesh, which presumably facilitates transfer of material to the duct.

A useful approach to localizing the arabinogalactan-proteins in non-woody tissues has been to treat the tissue with a mixture of a cationic detergent and an aldehyde fixative, followed by staining with the Yariv artificial antigen [174]. The specificity of the interaction has been discussed earlier. These red-coloured dyes precipitate arabinogalactan-proteins from plant extracts and are thought to stain arabinogalactan-proteins specifically in tissue sections.

Material staining specifically with the β-glucosyl substituted dyes, presumably arabinogalactan-protein, has been localized in a variety of plant tissues; it is associated with granules in the peripheral cytoplasm of cultured *Lolium* endosperm cells; with the aleurone layer of ceral seeds, especially at the cytoplasm-wall interface [13]; with vesicles in the intercellular spaces of cotyledon parenchyma cells of legume seeds [174]; with the cell membrane in leaf parenchyma cells of *Zantedeschia* and *Alocasia*; in secretory ducts of *Hedera helix* leaves [12]; associated with the stigma surface and style canal of *Gladiolus* [101, 259]. Material with similar staining properties has been found within the protoplast of pollen grains [260] and associated with the surfaces of plant protoplasts from both mono- and dicotyledonous callus cells [175, 176, 261). *Lolium multiflorum* endosperm protoplasts were strongly agglutinated by the IgA myeloma J539 protein, again indicating the surface localization of galactose containing molecules (Keller, F., pers. commun.).

To date no studies on the localization of the arabinogalactan-proteins at the ultra-structural level have been published but are a prerequisite to an understanding of their function.

FUNCTION

No general function can be ascribed to any of the arabinogalactans or arabinogalactan-proteins discussed. We can only speculate whether their ubiquitous distribution may indicate some fundamental role or whether they fulfill different functions in different situations. The basis for such speculation is not very solid since the information available regarding their localization at both the cellular and subcellular level is extremely limited and we have virtually no insights into the control of their biosynthesis.

The gums have been considered to be produced in response to fungal or bacterial infections, or to other pathological conditions. Bacteria are frequently asso-

ciated with *Acacia* gums even within the plant itself [282] where their presence may stimulate gum production. However, some trees produce gum while in an apparently healthy condition and since the composition, properties and structures of *Acacia senegal* gum appears to be independent of the nature of the wound or stimulus inducing gum production it does not seem necessary to propose that bacteria or other micro-organisms are obligatorily involved [149a]. It seems likely that the gum is the product of a normal metabolic process which is influenced by environmental conditions such as water stress and temperature [138, 262] and infectious agents [282]. The pronounced water-holding capacities of gum exudate gels have led to the suggestion that they function as a seal to isolate the infected or damaged area of the plant [139, 240, 149a]. Such a seal would prevent both spread of infection and moisture loss at the site of injury.

The present evidence suggests that gum formation is a local phenomenon although in view of the occurrence of arabinogalactan-proteins in the xylem sap of *Acer* [53, 90] their transport to exudate sites cannot be ruled out. The paucity of histological data on gum formation does not allow a resolution of the question of whether gums in general are secretory products or are products of tissue necrosis. However guggul [278] and probably the *Acacia senegal* gum [282] are secretory in origin and a recent study of gum production in carnation and elm in response to fungal infection has demonstrated that in these situations the gum is also a true secretory product [263]. An intense gummosis was observed in the xylem of these species, arising from an active secretion by cells associated with the xylem. The secretory product apparently flows through the pits into the xylem vessels to form a plug. The effectiveness of the arabinogalactans in forming plugs may be enhanced by their ability to form gel-like coacervates with other macromolecules [138]. The larch gum has been considered to be a secretion which is retained by the inner nonfunctioning part of the xylem, but why such enormous quantities of this gum should be deposited is a mystery. The wide variation in the concentration of this arabinogalactan both within individual trees, within the species and within the gymnosperms in general is not readily reconciled with any vital function [138].

In one situation, the style canal of *Lilium*, a nutritive role has been assigned to an arabinogalactan. Pollen which has been received and recognized as compatible at the stigma surface germinates and a pollen tube is produced which grows through the style canal. The canal is filled with a mucilage containing an arabinogalactan which provides carbohydrate precursors for the growing pollen tube cell wall [264].

The structural and physical properties of the arabinogalactan-proteins may indicate other likely roles. The molecules have a multi-branched bush-like form in contrast to other non-cellulosic cell wall polysaccharides which are essentially linear or sparsely branched. Their most striking properties are their adhesiveness and their ability to associate with other macromolecules. Perhaps, therefore, the adhesiveness is the key to the function. Arabinogalactans are known to be present in the pectic fraction [60, 161–166] and pectins occur in the middle lamella [162]. Thus it is possible that arabinogalactans may be present as adhesives in the middle lamella to cement cell–cell contacts. However no cytochemical examination of the middle lamella for the presence of arabinogalactan-proteins has been reported. The arbinogalactan-proteins may also be involved in the adhesion of callus cell clumps; suspension cultures of *Prunus avium* callus cells respond to an increase in temperature by an increase in the size of cell aggregates and there is a concomitant increase in the viscosity of the culture medium and the amount of secreted arabinogalactan (Raff, J., pers. commun.). But why should arabinogalactans be produced in some but not all tissue culture filtrates [112]?

One other situation where a general adhesive function has been considered is the receptive stigma surface. The development of a general 'stickiness' of the stigma surface with maturity of the flower has been demonstrated by its binding capacity for a number of proteins and glycoproteins. It has been suggested that the arabinogalactan-protein component of the surface secretion may be involved in capture and adhesion of pollen to the stigma surface [260].

The water holding capacity of the arabinogalactan-proteins may reflect other physiological roles: they may confer frost hardiness on particular plants [265]. Their ability to hydrate may also be important in their capacity to block wounds, and to resist drought. Under drought conditions certain algae retain their viability by secreting a gel layer which encloses the cell.

The localization of the arabinogalactan-protein at the protoplast surface [175, 176, 261] and the membrane-wall interface in intact cells [12, 13] suggests that they may be organized in some loose association with the membranes, indeed they may serve to protect membranes from desiccation. The finding of proteoglycolipids containing hydroxyproline in *Vicia faba* leaves [266] raises the possibility that they may also be associated with lipid. It is possible that arabinogalactan-proteins may be membrane bound as proteoglycolipids in a way analogous to the blood group glycolipids—with the protein or lipid component embedded in the double lipid bilayer and the hydrophilic carbohydrate component exposed at the extra-cytoplasmic face of the membrane.

Apart from the general adhesive and water holding properties, more specific interactions are possible. The molecules have the potential for two major types of interaction—a macromolecule–macromolecule association and a macromolecule–small ligand interaction. If the galactan backbone adopts either a helical or twisted triple helical configuration with the substituents oriented to the outside of the helix, several surfaces would be available for cooperative interactions—the regular areas of helix face, the helical grooves of the triple helix and the substituent chain. Specific interactions with carbohydrate-binding molecules or lectins of appropriate specificity could be envisaged. Although lectins with specificity for arabinose, rhamnose and the uronic acids have not been detected, they may well exist and be involved in specific recognition reactions. On the other hand, the arabinogalactan-proteins are themselves carbohydrate-binding macromolecules as shown by their ability to bind β-glycosyl Yariv antigens [11–13]. Since they are present on the outer plasmalemma surface of protoplasts [175, 176, 261] they must be considered as possible receptors for the β-glucan elicitors of the phytoalexin response in fungal infection, which have been shown to agglutinate potato leaf protoplasts [283].

It is possible that the outer chains, exposed at the molecular surface, are the functionally essential regions of the arabinogalactan-proteins—the inner protein and the galactan backbones serving only to present a variety of terminal substituents. This is the situation in the blood group active glycoproteins and glycolipids which have essentially the same saccharide structure with variations in the terminal saccharide sequences giving the molecule antigenic identity. Many of the analyses which led to our present understanding of the chemistry of blood group substances were performed on material secreted into pathologic ovarian cysts. The conceptual and technological gap in the realization that the same terminal disaccharide sequences were present in membrane glycolipids and glycoproteins took more than a decade to overcome. We may find a similar distribution of determinant groups in both secreted and membrane associated arabinogalactans. There is some evidence that the terminal disaccharides of some plant gums may have taxonomic significance [149], and an extension of this is that these groups may be implicated in the expression of identity of individual plants, tissues or cell types, just as the identity of animal cells is expressed by cell surface determinants such as the blood group and transplantation antigens. In this context it is interesting to note that the 1a region of the HLA complex is apparently specified by variations in terminal saccharide sequences [267] although other determinants are protein in nature [268]. In natural situations, tissues of different individuals are not usually grafted, but both plants and animals have the ability to reject foreign grafts [269]. The rejection mechanism is not as specific in plants as it is in animals, but, in general, grafts are tolerated between self or closely related species and are rejected when the grafting partners are genetically distant. The ability of plants to reject foreign grafts implies the presence of a mechanism for mutual recognition of the tissues involved [269, 270]. Perhaps then cell surface or secreted arabinogalactans are involved in this recognition and expression of identity through variations in the terminal saccharide sequences of proteoglycans or glycolipids which have common core structures.

All these speculations on the function of the arabinogalactan-proteins are founded on fragments of information drawn from diverse sources. Often, studies designed to answer questions quite unrelated to function have yielded some relevant information; we now have the technology and sufficient structural information available to initiate direct experimental approaches aimed at a definition of their function.

Molecules which may belong to the same class of arabinogalactans have been described from bovine lung [280] and from the cell surface of a trypanosomatid *Crithidia fasciculata* [281], indicating that the distribution of these polymers may not be restricted to the plant kingdom. For the present, however, we can classify the arabinogalactan-proteins as substances which are chemically well defined but which have not been assigned a biological role. The resolution of whether they are part of the 'ballast' of living organisms [274] or the 'garbage bin' of plant metabolism [275] or whether they have some fundamental significance, offers an exciting challenge for the future.

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