# Structural Characterization of Arabinan from *Pinus radiata* Cambium Tissue

**Zhenning Du**

**2000**

# Errata

| Page | Position | |
|---|---|---|
| 3 | Line 1 | Rosa glauca should read *Rosa glauca* |
| 4 | Line 15 | Pectin and Hemicellulose should read pectin and hemicellulose |
| 12 | Line 9 | Rosa should read *Rosa* |
| 13 | Line 27 | Rosa glauca should read *Rosa glauca* |
| 23 | Line 4 | (1998) should be placed after Youl *et al* |
| 34 | Fig. 2.2 | The X axis should read galacturonic acid |
| 42 | Fig. 2.4 | Acetaes should read Acetates |
| 44 | Line 13 | Fractins should read Fractions |
| 98 | Line 14 | Albersheim, P. *et al* should read Albersheim, P., An, J., Freshour, G., Fuller, M.S., Guillen, R., Ham, K.S., Hahn, M.G., Huang, J., O'Neill, M., Whitcombe, A. and Williams, M.V. |
| | Line 22 | rhamnogalacteronan should read rhamnogalacturonan |
| | Line 25 | *Multiflorum* should read *multiflorum* |
| | Line 31 | The issue number (No.2) should be deleted |
| 99 | Line 3 | pp.po-2 should read Abstracts (Posters), *p.* 2. |
| 100 | Line 3 | *Maximum* should read *maximum* |
| | Line 8 | Althaea officinalis should read *Althaea officinalis* |
| | Line 14 | lupinus albus should read *Lupinus albus* |
| 101 | Line 13 | The issue number (No.10) should be deleted |
| 103 | Line 6 | *Sylvestris* should read *sylvestris* |
| | Line 20 | The title of this paper should read: "An improved procedure for methylation analysis of oligosaccharides and polysaccharides." |
| 104 | Line 8 | The issue number (No.19) should be deleted |
| | Line 12 | *Phytophtora* should read *Phytophthora* |
| | Line 18 | *larix* should read *Larix* |
| 105 | Line 32 | *Napus* should read *napus* |
| 106 | Line 16 | Universidy should read University |
| | Line 27 | monosaccharide should read nonasaccharide |
| 108 | Line 5 | protopedtin should read protopectin |
| | Line 10 | Actinia deliciosa should read *Actinidia deliciosa* |
| | Line 17 | Abies balsamea should read *Abies balsamea* |
| | Line 21 | Abies balsamea should read *Abies balsamea* |
| | Line 31 | Solanum tuberosum should read *Solanum tuberosum* |
| 109 | Line 17 | Brassica campestris should read *Brassica campestris* |
| | Line 26 | This reference is to Fry SC and not Stephen CF |
| | Line 29 | Brassica olearacea var capitata should read *Brassica oleracea var capitata* |
| | Line 32 | Brassica oleracea should read *Brassica oleracea* |
| 110 | Line 2 | Vinca rosea should read *Vinca rosea* |
| | Line 25 | No. should be deleted |
| | Line 36 | Aspergillus niger should read *Aspergillus niger* |

# Structural Characterization of Arabinan from *Pinus radiata* Cambium Tissue

A thesis presented in partial fulfilment of the
requirements for the degree of

Master of Science
in
Biochemistry

at Massey University, Palmerston North
New Zealand

**Zhenning Du**

**2000**

# ABSTRACT

Cambium tissue isolated from *Pinus radiata* was frozen, thawed, washed with cold phosphate buffer, then extracted with phosphate buffer by passage through a French pressure cell. The washings and French press extract were dialyzed.

The pectic components partially purified by chromatography on DEAE-cellulose. These polymers bound weakly to DEAE-cellulose at pH 8.0. In both extracts, an early acidic peak of high arabinose and galactose content was separated from a late acidic peak with mostly uronic acid. Within the early acidic peak, successive fractions showed an increase in uronic acid and rhamnose content.

Treatment of the extracts with boiling water caused a decrease in binding of the arabinose-rich polymer to the DEAE cellulose reflected by earlier elution, and a minor decrease in average molecular size, consistent with β-elimination of the pectic backbone.

The acidic fractions were then further fractionated by gel filtration on Superose and Superdex FPLC columns. The earliest of these acidic fractions had ratios of Ara:Gal:Rha = 30:16:1, this suggesting relatively long side chains of about 46 residues, and were mostly excluded by the Superdex-peptide column.

The native molecular mass of the arabinose-rich polymers was determined by size exclusion chromatography and molecular masses of extract A and B were observed to be 40-100 kDa and up to >500 kDa, respectively by Superose and Superdex FPLC columns.

Other characterization studies on the arabinan involved quantitative analysis of the monosaccharides derived by sulfuric acid hydrolysis and quantitation of the sugar linkages using methylation analysis. Methylation analysis suggested a highly branched arabinan structure and the presence of terminal galactosyl residues on a branched arabinan core.

From the results, a tentative structure has been suggested for the arabinose rich polymers. Arabinose was mainly present as 3-, 5-, 3,5-, and terminal linked residues. Galactose was mainly present as terminal residues.

# ACKNOWLEDGEMENTS

I would like to first express sincere appreciation and gratitude to my supervisor, **Dr Ian Andrew** (Institute of Molecular BioSciences, Massey University), for excellent supervision and encouragement throughout all stages of this project. Without his persistent support in both my research project and my personal life, it would have been impossible for me to complete this work.

I am very grateful to **Dr Erin M. O'Donoghue** (Crop & Food Research Institute), for her support, guidance, and helpful comments.

I would like to thank all those academic, technical and administrative staff of the Institute of Molecular BioSciences, Massey University, who helped me on various occasions. Special thanks to **Dr Peter Farley** and **Dr Deming Gong** for their kindness whenever I came to them with questions, **Mr Ruwan Dissanayake, Mr Adrian Koolaard, Mr Chris Burrows** and **Ms Carole Flyger** for their willing help in the laboratory.

I wish to express my appreciation to the New Zealand Institute of Chemistry for providing a conference grant.

Finally, a special thank you to my wife Ningxia and my daughter Jiaqi for their love and excellent support throughout.

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# LIST OF ABBREVIATIONS

| | |
|---|---|
| $A_{400/450nm}$ | Absorbances at 400 and 450nm |
| AG | Arabinogalactan |
| AGPs | Arabinogalactan proteins |
| Ara | Arabinose |
| °C | Degrees celsius |
| ca. | Approximately |
| DEAE | Diethylaminoethyl |
| DMSO | Dimethyl sulphoxide |
| EDTA | Ethylenediaminetetraacetic acid |
| ETOH | Ethanol |
| FID | Flame-ionization detector |
| FPLC | Fast protein liquid chromatography |
| Gal | Galactose |
| GalA | Galacturonic acid |
| GC | Gas chromatography |
| Glc | Glucose |
| HCl | Hydrochloric acid |
| HOAc | Acetic acid |
| HRGPs | Hydroxyproline-rich glycoproteins |
| kDa | Kilodaltons |
| Man | Mannose |
| MS | Mass spectrometryMillilitery |
| NMR | Nuclear magnetic resonance |
| pH | $-Log[H^+]$ |
| RG | Rhamnogalacturonan |

| | |
|---|---|
| Rha | Rhamnose |
| TFA | Trifluoroacetic acid |
| TMSI | Trimethylsilyl-imidazole |
| UV | Ultra violet light |

SI units (System International) are not included in the list above.

# 1. Chapter One: Introduction

## 1.1. Arabinans in *Pinus radiata* Cambium Tissue, Merit for Study

It is hard to say for certain just how or when *P. radiata* was first introduced into New Zealand. Weston (1957) speculated that miners who traveled the world from one gold rush to another in the early years of the 19th century might have introduced the seed from California, USA, to Australia, and thence to New Zealand. Kininmonth and Whitehouse (1991) suggested that some wealthy settler who wished to create park-like surroundings in his new environment shipped out the first seedlings from England (where radiata pine had been grown since the early 1830s). Whatever the case, a number of importations of both seedlings and seed, mainly from Australia, had ensured that *P. radiata* was well established in New Zealand since 1865 (Weston, 1957).

The Royal Commission on Forestry of 1913 recommended *P. radiata* as the most suitable of the introduced tree species for extensive planting in New Zealand because of its ease of propagation, rapid height growth, high volume production, adaptability to a wide range of sites, and unique wood quality. Since then, there have been two major periods of intensive plantation development in New Zealand. The first time was from the 1920s to early 1930s. The second time was from the early 1960s until the present day. Today it is the most important plantation forestry species in New Zealand and makes a significant contribution to the New Zealand economy. Although it occupies only 4.5% of the land area of New Zealand, it accounted for most of the domestic demand for forest products and provided 13.5% of New Zealand's total export earnings (NZ$ 2.6 billion) in the year to March 1994 (Walter and Smith, 1995). This figure is predicted to increase to perhaps 30% by the year 2010 (Ministry of Forestry, 1990).

The successful plantation of *P. radiata* in New Zealand is not only a result of a climate which benefits tree growth, but also a result of innovative management, tree improvement practices and a lot of research work including plant biochemistry research. For example, Linder and Rook (1984) state that there are basically two ways of increasing yield from trees:

- Increase the net uptake of carbon dioxide, thus increasing total carbohydrate production; and

- Channel more of these resources into stem growth, either by silviculture or by genetic selection.

In all plants that contain specialized tissues, growth and formation of new cells are localized in regions of cell division known as meristems. Meristems that build up the fundamental structure of a plant are called primary meristems. The growing points of a tree, which gives rise to stem, branches and roots, contain apical meristems, and the vascular cambium and phellogen, which produce radial growth, are lateral meristems (Kininmonth and Whitehouse, 1991).

Iqbal (1995) considered the concept of the "cambium" in the following: "While every vascular plant requires at least a shoot meristem, the cambium (a lateral meristem) is not indispensable. The cambium develops from provascular elements between phloem and xylem of the primary vascular system, forms a continuous sheath around the xylem core of stems and roots, and extends in the form of strips into leaves if they have secondary growth." The cambium acts as a meristematic template for the secondary vascular tissues (xylem and phloem) and by doing so bridges the gap between these tissues. Therefore, the cambium is supposed to play an active physiological role even when in the dormant stage (Riding and Little, 1984, 1986).

The *P. radiata* cambium wall differs from previously studied primary walls in its higher lignin content and in the rather tight binding of much of the pectic material within the wall (Andrew and McKee, unpublished). Tracheid length and wood quality is dependent on the cambium. Therefore the primary wall structure, the polymers, and polymer cross-linking, which may affect growth and elongation, are important in providing a basis for improving wood quality through environmental and hormone regulation. This cannot be fully understood until the polysaccharides of the primary wall and their structures have also been characterized.

A notable feature of the *P. radiata* cambium tissue is its abundant water-soluble arabinan (Andrew and McKee, 1997). Arabinan occurs in various plant materials, notably in seeds, fruits, and roots. Isolation and investigation of arabinan structures from cotyledons of red gram (Swamy and Salimath, 1991), roots of marsh mallow (Capek *et al.,* 1983), roots of horsebean (Joseleau *et al.,* 1983), cabbage (Stevens and Selvendran, 1980), grape juice (Villetaz *et al.,* 1981), apple juice (Churms *et al.,* 1983),

and inner bark of Rosa glauca stems (Joseleau *et al.,* 1977) have been described. Arabinans are generally associated with pectins.

In *P. radiata*, wood quality and the resistance of leaves and wood to infection by pathogens are determined by a number of factors, important among which is the nature of the constituent polysaccharides. These polysaccharides include the pectic polysaccharides, which are major constituents of the cell walls of many higher plants and play an important role in both the elongation of plant cells and defense against invading fungal pathogens.

Pectin is believed to control the porosity of walls. It allows smaller molecules to enter the wall and possibly the cell. Disruption of the pectin alters the porosity (Andrew, *pers. comm.*). Pectin is important as a first line of defense against pathogens (fungi, bacteria). Fungi and bacteria have pectin enzymes and can degrade pectin to oligogalacturonides. These oligogalacturonides are "elicitors." They elicit a defense response, which prevents the further invasion of the pathogen (Davis *et al.,* 1986a; 1986b; Davis and Hahlbrock, 1987).

Although we have some understanding of the function of pectin, the role of arabinans in seeds and plant cell walls is unknown. To date, arabinans have not received much attention although they have some interesting physicochemical properties (Cros *et al.,* 1994).

Some preliminary work done in our laboratory suggests that *P. radiata* arabinan differs markedly from most other arabinans studied in that, as well as 1→5-linked residues, it has high levels of 1→3-linked and 1→3,5-branched residues (Little *et al.,* 1980; Fenemor, 1984; Andrew and McKee, 1997; Andrew and Little, 1997). The arabinan is present not only in cell walls, but also as soluble polysaccharides in the cells.

## 1.2. The Constituents of Cell Walls

The plant cell is surrounded by a cell wall, which serves as protection against injury such as desiccation and osmotic shock (Duffus and Duffus, 1984). There are two general types of plant cell walls: primary and secondary. Primary cell walls are first laid down by young, undifferentiated cells that are still growing. The primary cell walls provide the first barrier to pests; they physically control the rate of cell growth and

form the basic structural backbone of growing plant cells and tissues. These walls can best be described as reinforced multi-component gels (Bacic *et al,* 1988). Secondary cell walls, as distinct from the primary cell walls, are formed by cells that have stopped or are stopping growth and that are differentiating into cells with specialized functions (Darvill *et al,* 1980).

Primary cell walls are composed of cellulose fibrils embedded in relatively large amounts of an amorphous mixture of polysaccharides and glycoproteins. Cellulose is relatively more abundant in secondary cell walls, and these walls often contain significant amounts of lignin. Cells no longer grow once lignin is added to their walls (Albersheim *et al,* 1996). Generally, the polymers of primary cell walls are easier to isolate than those of secondary cell walls (York *et al,* 1985).

The major classical cell wall fractions have been described as follows (Preston, 1974):

- Pectic substances, which are extracted by boiling water and hot ammonium oxalate.
- Hemicelluloses, which are extracted usually by 4N KOH at room temperature.
- Cellulose, the residue after Pectin and Hemicellulose extraction and often extracted with 72% sulfuric acid.
- Protein which is often removed with polysaccharide fractions.
- Lignin which is removed by acid-chlorite treatment.

Cellulose, hemicellulose, pectic polysaccharide, structural protein, and lignin have been identified as the major components of the plant cell wall. Secondary cell walls have greatly increased levels of cellulose together with hemicellulose and lignin.

Albersheim *et al* (1994) reported that primary cell walls are composed of about 20% cellulose microfibrils, 70-80% non-cellulosic polysaccharides, and up to 10% structural glycoproteins. Structural elucidation of primary cell wall polymers is essential to dissecting and understanding their roles in wall structure and other physiological functions.

Because the study of arabinans is related to the pectic polysaccharide and protein, they will be specifically discussed in the following sections. Cellulose and hemicellulose

will be described only briefly, while lignin is a characteristic component of secondarily thickened walls and is therefore not further discussed.

### 1.2.1. Cellulose

Cellulose is the fibrillar component and forms the basic structure of cell walls in all higher land plants. It usually increases from about 20-30% of the dry weight of the primary cell wall to 40-90% of the secondary cell wall (Stephen, 1988). All primary cell walls depend on cellulose microfibrils for tensile strength. In order for primary cell walls to grow, the cellulose microfibrils must move relative to one another and the microfibrils must be kept from combining to form large, intractable aggregates of the type present in secondary cell walls (Albersheim *et al.*, 1996).

Chemically, cellulose is a simple linear polymer of β-(1→4)-linked glucopyranosyl residues. Each glucose residue is rotated at 180° relative to its nearest neighbor giving a repeat unit structure of cellobiose, and glucan chains exist as extended ribbons with a 2-fold screw axis stabilized by intramolecular hydrogen bonding. (Bacic *et al.,* 1988). The width, degree of polymerization, and crystallinity of the cellulose microfibrils are highly variable and depend on the source and age of the tissue. The microfibrils are highly crystalline and make up an important part of the framework of the cell walls of all higher plants (Talmadge *et al.,* 1973). The degree of polymerization of the primary wall cellulose is relatively low and heterogeneous (2,000-6,000) however that of the secondary wall cellulose is higher and more homogeneous, about 13,000-16,000 (Cote, 1977).

### 1.2.2. Hemicelluloses

Hemicelluloses are defined as alkali-soluble cell wall polysaccharides, so named because of their close association with cellulose and because they were once thought to be cellulose precursors. Hemicelluloses bind tightly, via multiple hydrogen bonds, to the surface of cellulose microfibrils. It is believed that the hydrogen bonding to the cellulose microfibrils by the cellulose-like backbone of a hemicellulose chain is eventually sterically interrupted by carbohydrate side chains of the hemicellulose (Levy *et al.,* 1991). For the most part, hemicelluloses are composed of D-glucose, D-mannose, D-xylose, L-arabinose residues joined together in different combinations and in various

glycosidic linkages to give the hemicellulose polysaccharides (Ericson and Elbein, 1980). The nature of the hemicelluloses isolated from different tissues varies considerably. Hemicelluloses of the secondary cell walls have been divided into two broad groups: the glucomannans and xylans, whereas the xyloglucan, arabinoxylan and mixed linked glucans are components of all primary cell walls, although the relative amounts of these hemicelluloses varies from plant to plant (Albersheim *et al.,* 1996, Fenemor, 1982).

Variations on basic structure render it valuable to discuss particular hemicellulose types individually.

**a. Glucomannans and Galactoglucomannans.** These polysaccharides occur in significantly larger proportions in the lignified secondary cell walls of coniferous gymnosperms (12-15%) than angiosperms (3-5%) (Bacic *et al.,* 1988). They consist of chains of randomly arranged D-glucose and D-mannose residues linked β-(1→4) in the main chain.

The glucomannans are the main non-cellulosic polysaccharides present in cell wall of mature softwood and they tend to be insoluble in water and if resistant to weak alkaline extraction they can be extracted with alkali-borate solution (Northcote, 1972), and subsequently fractionated with $Ba(OH)_2$. These polysaccharides are structurally similar to cellulose and seem to be closely associated with the cellulose molecules in the cell wall. For gymnosperms, the glucose to mannose ratio is approximately 1:3, whereas in angiosperms the ratio is approximately 1:2 (Bacic, *et al.,* 1988).

The water-soluble galactoglucomannans are isolated in low yields from softwoods. The galactoglucomannans are a homogeneous polymer and usually have a galactose: glucose: mannose ratio of 1:1:3 (Timell, 1965). The structure is the same as the alkali-soluble glucomannans, the main difference being the increase in terminal side chain galactose residues, which may account for the water-soluble properties of the polymer.

**b. Xylans and Arabinoxylans.** The xylans are abundant noncellulosic polysaccharides in the majority of angiosperms where they account for 20-30% of the dry weight of woody tissues (Aspinall, 1980). They are mainly secondary wall

components but they are also found in the primary walls of some plants. In gymnosperms, where galactoglucomannans and glucomannans form the major hemicelluloses, xylans are less abundant (8%) and more difficult to isolate in pure form (Timell, 1965). The general structure of xylans is that of an essentially linear main chain of D-xylopyranose residues joined by β-(1→4) links. The xylans have a degree of polymerization of 150-200. Attached to these chains are L-arabinofuranose, D-glucuronic acid, or its 4-methylether and D-galactose. L-Arabinosyl units are generally furanosyl, (1→3)-linked to the xylan, while uronic acid residues may be linked through (1→2) or (1→3) links to the main chain. The (1→2) linkage is the usual form (Timell, 1965). Xylans in hardwoods generally lack arabinose, but instead commonly bear acetyl groups at C-3 of the xylose residues. The modes of attachment of single unit (4-*O*-methyl-) D-glucuronic acid and L-arabinofuranose residues are well established for many xylans, but the lengths and exact structures of the more extended sidechains remain to be established with precision (Timell, 1964; 1965).

An acidic arabinoxylan is a major hemicellulose present in monocot seedling primary cell walls (Darvill *et al.*, 1978; Buchala, 1974; Wada and Ray, 1978). These arabinoxylans always contain a linear (1→4)-linked xylan backbone. Similar arabinose-rich arabinoxylans have been isolated from barley aleurone cell walls (McNeil *et al.*, 1975) and found in several species of cultured monocot cells (Burke *et al.*, 1974). Arabinoxylans have been detected in the extracellular polysaccharides of sycamore, sugar cane, wheat, tobacco and periwinkle (Keegstra *et al.*, 1973; Burke *et al.*, 1974; Akiyama and Kato, 1982; Takeuchi and Komamine, 1980b), and an arabinoxylan has been isolated in a highly pure form from tobacco extracellular polysaccharides (Akiyama *et al.*, 1984). This xylan (4% arabinosyl and 89.5% xylosyl residues) was purified first by anion exchange and then by cellulose chromatography.

c. **Mixed-Link Glucan.** Glucans consisting of a mixture of β-(1→3)-linked and β-(1→4)-linked glucosyl residues have been observed in primary cell walls of several plants. These mixed-link β-glucans have been found in nearly all of the cell wall preparations obtained from monocots, and are thought to be important structural matrix components (Darvill *et al.*, 1980; Fenemor, 1982). The mixed-link

glucans comprise a family of polymers, which are heterogeneous with respect to molecular size and fine structure, varying with tissue, age, and source. They generally contain approximately 30% (1→3)-and 70% (1→4)-β-D-glucosidic linkages with more than 90% of the molecule consisting of (1→3)-β-D-linked cellotriosyl and cellotetraosyl residues (Woodward *et al.,* 1983a,b; Kato and Nevins, 1984a,b). Individual (1→3)-and (1→4)-linked residues, blocks of more than three contiguous (1→4)-linked residues, and regions of contiguous (1→3)-linked residues may also be present.

Woodward *et al.* (1983a) reported that the 40°C water-soluble (1→3, 1→4)-β-D-glucan from barley endosperm walls contains runs of between 4 and 14 contiguous (1→4)-linked but no contiguous (1→3)-residues. In contrast, water-soluble and water-insoluble (1→3, 1→4)-β-D-glucans of *Zea mays* shoot walls contain regions of two, three, or four contiguous (1→3)-linked residues as well as blocks of more than three contiguous (1→4)-linked residues (Kato and Nevins, 1984a,b).

Fincher and Stone (1986) showed that the molecular weight estimates for (1→3, 1→4)-β-D-glucans range from 20,000-1,000,000. In previous reports (Igarashi and Sakurai, 1965; Woodward *et al.,* 1983b), however, where determined by sedimentation equilibrium ultracentrifugtion, they are in the range of 200,000-300,000 corresponding to the degree of polymerization of 1200-1850. The (1→3, 1→4)-β-D-glucans are asymmetrical molecules assuming an extended conformation in aqueous solution.

**d. Xyloglucans.** Xyloglucans are major hemicelluloses of dicot primary cell walls and perhaps the most thoroughly understood of the noncellulosic polysaccharides (Bauer *et al.,* 1973). Many of these polysaccharides have been detected by the formation of a characteristic blue stain on reaction with iodine. The resulting use of the term amyloid is misleading in implying a nonexistent structural relationship to amylose (Aspinall, 1980).

Xyloglucan is the predominant hemicellulose in the primary wall of *P. radiata* as well as of dicots. It is believed to cross-link cellulose microfibrils and to establish a strong 3-dimensional network (Fry, 1989). The actual evidence for this model is currently being hotly debated (Melton *et al.,* 1997). Xyloglucan is envisaged as the

load-bearing component of the wall, and extension growth involves wall loosening which has been suggested to be due to a turnover of xyloglucan. Xyloglucan turnover is one of the early events associated with auxin-stimulated growth.

Xyloglucan resembles cellulose but the chains are much shorter and they are substituted. Turnover is catalyzed by several enzymes and involves changes in molecular weight. One of the breakdown products of xyloglucan is a nonamer called XXFG, a potent oligosaccharin, or bioactive oligosaccharide. It is an auxin antagonist at nanomolar concentrations, and may have a regulatory role during growth. (McDougall and Fry, 1991).

The structure of the xyloglucans has been determined by a combination of methylation analysis and chromatographic separation of the oligosaccharides produced by partial enzymatic digests (Darvill *et al.,* 1980). Members of the xyloglucan family have a linear extended backbone of (1→4)-linked β-D-glucopyranosyl residues substituted at C(*O*) 6 with α-D-xylopyranosyl residues. However, some heterogeneity in their fine structure exists. The heterogeneity resides in the substituents on the xylopyranosyl residues and includes β-D-galactopyranosyl-(1→2)-, α-L-arabinofuranosyl-(1→2)- and α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-side chains (Darvill *et al.,* 1980; McNeil *et al.,* 1984).

The molecular weight of xyloglucans from different plants and even within the same plant varies widely from 7,600 for the heteroglucan from sycamore suspension culture medium to 180,000 for the heteroglucan from walls of suspension cultured soybean cells (Bauer *et al.,* 1973; Hayashi *et al.,* 1981). Xyloglucans may interact with other wall components either by noncovalent (hydrogen bonding to cellulose) or possibly covalent interactions (with pectic polysaccharides). In previous reports, it was demonstrated that xyloglucan was also the major hemicellulose of the *P. radiata* hypocotyl cell walls. From the results, a tentative structure has been suggested for the xyloglucan, which showed that this component was a fucogalactoxyloglucan of similar structure to those reported from dicots (Andrew and Little, 1997).

## 1.3. Pectic Substances

Pectic substances are a group of closely associated polysaccharides from the primary cell walls and intercellular regions of higher plant (Voragen *et al.,* 1995). They are deposited mainly in the early stages of growth when the area of the wall is increasing. The functions of pectin are diverse and include ill-defined roles in cell expansion, cell adhesion, cell wall porosity, and plant development and defense (Carpita and Gibeaut, 1993; Jarvis, 1984). The pectic polysaccharides are probably the most complex class of wall polysaccharides (Bacic, 1988) and comprise a family of acidic polymers like homogalacturonans, rhamnogalacturonans with several neutral polymers like arabinans, galactans and arabinogalactans attached to it. They are usually extracted from walls with water or aqueous solutions of chelating agents such as ethylenediaminetetraacetic acid (EDTA) or ammonium oxalate, and purified preparations of polysaccharide hydrolases have also been used for extraction (Bacic, 1988; Voragen *et al.,* 1995; O'Neill *et al.,* 1990).

The pectic polysaccharides are built on a pectin backbone, i.e. a polymer of galacturonic acid, which may be methylesterified or bound to calcium. Rhamnose residues occur in the chain, usually in blocks, so we get galacturonan blocks and rhamnogalacturonan blocks. Pectic polysaccharides may form a 3-D network interwoven with the cellulose-xyloglucan network. The structural pectic polysaccharides are summarized in the Figure 1.1.

From studies on pectins from many sources, it has become clear that pectin is not a homopolysaccharide (Voragen *et al.,* 1995). Pectins are esterified galacturonans or more commonly rhamnogalacturonans in which the α-(1→4)-linked D-galacturonan chains are interrupted at intervals by the insertion of α-L-rhamnopyranose residues that carry arabinan or galactan side chains. Other constituent sugars are attached in sidechains and include D-galactose, D-xylose, and, less frequently, L-fucose, D-glucuronic acid, the rather rare sugars, 2-*O*-methyl-D-xylose, 2-*O*-methyl-L-fucose, and the branched chain sugar D-apiose. Most of these sugars occur in short sidechains, but the more common neutral constituents, D-galactose and L-arabinose, are also found in multiple units. At the same time, account must be taken of the fact that arabinans, and galactans or arabinogalactans may be isolated from pectin-rich sources (Aspinall, 1980).

GalA—$^{4}$(GalA)$_{n}$—$^{2}$ Rha—$^{4}$ GalA—$^{2}$ Rha—$^{4}$ GalA—

Galacturonan backbone — Rhamnogalacturonan backbone

**Galacturonan backbone:**

**Homogalacturonan** modified by:
Acetyl substituents
Methyl-esterification

**Xylogalacturonan** **(Xyl on 3-)**

**Apiogalacturonan** **(Api on 2-)**

**Rhamnogalacturonan II** **(complex)**

**Rhamnogalacturonan backbone (GalA-Rha)$_{n}$:**

**Rhamnogalacturonan I** **(on Rha 3-):**

GalA—$^{2}$Rha$^{4}$—Ara$^{5}$—Ara—
/
GalA
/
Rha—GalA—$^{2}$Rha$^{4}$—Gal$^{4}$—Gal—
/

**Arabinan** (mostly 5-linked)

**Galactan** (mostly 4-linked)

**Arabinogalactan** (Type I: Gal 4-linked)
(Type II: Gal 3,6-linked)

**Figure 1.1 Pectic Polysaccharides**

The arabinans, whether occurring as side chains to pectins or as polymers associated with pectin, mostly consist of (1→5)-linked α-L-arabinofuranosyl residues.

Individual classes of the pectic substances will be discussed below.

### 1.3.1. Homogalacturonans

Homogalacturonans are defined as polymers consisting predominantly of α-(1→4)-linked galacturonosyl residues (McNeil *et al.,* 1984). They have been isolated from various plant tissues such as sunflower heads and seeds (Zitko and Bishop, 1965; 1966), sisal (Aspinall and Rodrigues, 1958), rice endosperm cell walls (Shibuya and Nakane, 1984), suspension cultured primary cell walls from Rosa (Chambat and Joseleau, 1980) and sycamore (McNeil et al., 1984), and from apple pectin (Barrett and Northcote, 1965).

Homogalacturonans are usually extracted from plant material by mild acid treatment (Zitko and Bishop, 1966; Thibault *et al.,* 1993). Depending on the extraction method used, some modification of the polymer may occur. Uninterrupted homogalacturonan regions with a degree of polymerisation of approximately 70-100 have been isolated from various plant tissues like carrot (Konno, 1986), apple, beet and citrus (Zitko and Bishop, 1966).

An important feature of galacturonans is the esterification of the galacturonic acid residues with methanol and /or acetic acid (Schols and Voragen, 1996). Many (~70%) of the carboxyl groups of the galactosyluronic acid residues of primary cell wall homogalacturonan are methyl-esterified. The degree of methyl-esterification is variable (Voragen *et al.,* 1995; Morris, 1986; Williamson *et al.,* 1990). It is possible that the degree of esterification of homogalacturonans decreases in proportion to the amount of time the homogalacturonan is resident in the wall. The variously esterified forms of homogalacturonan appear to be concentrated in specific regions of primary cell walls (McCann *et al.,* 1994). For example, the middle lamella appears to contain sparsely methyl-esterified homogalacturonan (Moore *et al.,* 1986; Geitmann *et al.,* 1995). Homogalacturonans that are less than ~50 % methyl-esterified readily form gels, especially in the presence of calcium, which is present in primary cell walls. The fewer the methyl ester groups and the more the distribution of methyl esters is in blocks, the

greater the propensity of the homogalacturonan chains to form gels (Morris, 1986; Williamson *et al.,* 1990). Homogalacturonans are also modified by acetyl substituents. Acetyl groups are usually only present in low amounts in pectins from e.g. apple and citrus, but are present in much higher amounts in pectins from sugar beet (Rombouts and Thibault, 1986) and potato (Voragen *et al.,* 1986a).

### 1.3.2. Rhamnogalacturonan

**a. Rhamnogalacturonan I.** Rhamnogalacturonan I (RG-I) has a backbone composed of as many as 100 repeats of the disaccharide [→2)-α-L-rhamnosyl- (1→4)-α-D-galactosyluronic acid-(1→] (McNeil *et al.,* 1980; Lau *et al.,* 1985). Arabinosyl- and galactosyl-rich side chains are attached to *O*-4 of the rhamnosyl residues, although the proportion of rhamnosyl residues with attached side chains varies from ~20% to ~80% depending on the source of the polysaccharide. By specific degradation of the uronic acids with a lithium treatment (Mort and Bauer, 1982; Lau *et al.,* 1988a), the presence of at least 30 different side chains has been indicated. Some side chains consisted of at least 15 glycosyl residues (McNeil *et al.,* 1980; 1982; Lau *et al.,* 1988b). Approximately one half of the (1→2)-linked-L-rhamnosyl residues are branched at *O*-4 with side chains averaging about seven glycosyl residues in length composed of D-galactosyl and L-arabinosyl units. Small amounts of L-fucosyl groups have been found linked to L-rhamnosyl units (McNeil *et al.,* 1980). Occasionally, the side chains are terminated by glucuronosyl, or 4-*O*-methyl glucuronosyl residues and xylosyl residues always account for about 1% of RG-I, but their locations in the molecule have not been ascertained (An *et al.,* 1994). The xylosyl residues may be attached to the galactosyluronic acid residues of the backbone. On the basis of their sugar and glycosyl residues, it appears that pectin fragments similar to RG-I are present in the cell walls of many higher plants. For example, suspension-cultured rice and corn (Thomas *et al.,* 1989), Douglas fir (Thomas *et al.,* 1987), Rosa glauca (Chambat *et al.,* 1984), carrots (Konno *et al.,* 1986), kiwi fruit (Redgwell *et al.,* 1988), tomato tissue (Pressey and Himmelsback, 1984), rice endosperm (Shibuya and Nakane, 1984), apple tissue and pectins (Voragen *et al.,* 1986b; Renard, *et al.,* 1991; De Vries *et al.,* 1982), and sugar beet pulp and pectins (Raynal *et al.,* 1991; De Vries *et al.,* 1982), although the nature and quantity of the side chains vary. Much remains to be learned about the

structures and distribution of the side chains of this large family of polysaccharides (Albersheim *et al.*, 1996).

**b. Rhamnogalacturonan II.** Rhamnogalacturonan II (RG-II) is a quantitatively minor component of plant cell walls and has an extremely complex structure (Voragen, 1995). RG-II has a backbone of galacturonic acid, with side chains containing rhamnose and a number of other sugars.

RG-II is a low molecular weight (~4.8 kDa) complex polysaccharide, which has 11 different sugars in more than 20 different linkages (O'Neill *et al.*, 1990). The structure of RG-II is highly conserved, as apparently identical RG-II molecules have been obtained following *endo*polygalacturonase treatment of the primary cell walls of rice, onion, Douglas fir, sycamore, grape, and apple, and strong evidence of the presence of this polysaccharide has been obtained in other cell walls (Albersheim *et al.*, 1996).

RG-II is separated from the other pectic polysaccharides solubilized by the *endo*polygalacturonase by anion exchange and gel permeation chromatography. RG-II has a 'homogalacturonan' backbone composed of about nine (1→4) linked α-D-galactosyluronic acid residues (Whitcombe *et al.*, 1995). The number of residues in the backbone may depend on the particular glycosidic bond of homogalacturonan that the endopolygalacturonase cleaves when solubilizing RG-II. It is believed that the homogalacturonan is covalently linked to the *O*-1 and /or *O*-4 of the terminal reducing and non-reducing galactosyluronic acid residues of the galacturonan backbone of RG-II.

The elucidation of the primary structures of RG-I and RG-II has been summarized by O'Neill *et al.* (1990). RG-II was found to consist of approximately 30 glycosyl residues, among them the rare sugars mentioned earlier. It contains a high proportion of rhamnosyl residues, which occur (1→3) and (1→2,3,4) linked and as terminal units, in contrast to rhamnogalacturonan I where they are (1→2) and (1→2,4) linked (Voragen, 1995).

There are four different complex side chains, which are attached to *O*-2 or *O*-3 of four of the backbone residues and two of these chains start with apiose. These side chains sterically prevent *endo*polygalacturonase from cleaving the backbone, which

explains why intact RG-II is released from cell walls by *endo*polygalacturonase. Indeed, RG-II is highly resistant to glycanase digestion (Darvill *et al.,* 1980). Ishii *et al.* (1999) reported that RG-II forms a borate ester crosslinked dimer, which involves apiose of each monomer. RG-II binds heavy metals, which can then increase rate of dimer formation with borate. In the cell wall, RG-II is bound to homogalacturonan, and probably constrained to favor dimer formation. In RG-II, boron links two RG-II molecules via a borate diester bond. Dimeric RG-II is stable at low pH. The widespread occurrence and conserved structure of RG-II have led to the suggestion that borate ester cross-linked RG-II is required for the development of a normal cell wall. These cells have a high specific-growth rate, no significant lag phase, and reproducible changes in their wall pore size during the transition from the growth phase to the stationary phase (Fleischer, *et al.,* 1998). RG-I and RG-II both contain rhamnosyl and galactosyluronic acid residues, but these polysaccharides are not structurally related.

### 1.3.3. Galactan

Galactans have been isolated from citrus pectin (Labavitch *et al.,* 1976), white willow (Toman *et al.,* 1972), and beech (Meier, 1962). The pectic galactans are primarily β-(1→4)-linked polymers. The 4-linkage has been established by methylation analysis. The galactosidic linkages were shown to be in the β-anomeric configuration by the fact that these linkages are susceptible to hydrolysis by an endo-1-4-β-galactanase and by their low positive optical rotation (Labavitch *et al.,* 1976). Furthermore, oligosaccharides produced from the intact galactan by partial acid hydrolysis (Toman *et al.,* 1972) are susceptible to further hydrolysis by a β-galactosidase. Finally, the β-configuration of some of the galactosidic linkages of oligosaccharides derived from a galactan by partial acid hydrolysis (Meier, 1962) has been established by chromatographic comparison to known standards.

No homogalactan has been isolated directly from a primary cell wall, although analysis of the primary walls of suspension-cultured sycamore cells (Talmadge *et al.,* 1973) has indicated the probable presence of β-(1→4)-linked D-galactan chains in neutral pectic side chains. Those galactans that have been studied have degrees of polymerization ranging from 33 (Toman *et al.,* 1972) to 50 (McNeil and Albersheim, 1975). These

values were obtained by vapor pressure osmosis and by comparing the ratio of terminal to internal sugars as obtained by methylation analysis.

β-(1→4)-linked D-galactans, devoid of arabinose residues but containing 5-10% of uronic acid (glucuronic and /or galacturonic acid) residues, are found in compression wood of gymnosperms, for example, red spruce (Schreuder *et al.,* 1966) and tamarack (Jiang and Timell, 1972). In these polysaccharides, it is likely that the uronic acid residues are end groups.

The β-(1→4)-linked D-galactan from citrus pectin is a commercial product (Evans and Linker, 1973), and similarly constituted polysaccharides have been extracted from seeds, leaves, bark, tubers and, in crude form, from delignified compression wood. The structure is shown in the Figure 1.2.

→4)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→

**Figure 1.2 Structure of Galactan. *Galp*, Galactopyranose (From Stephen, 1983)**

Darvill *et al.* (1980) showed that galactans have been obtained which contain 6-linked galactosyl residues in addition to 4-linked residues. In two of the cases studied, the 6-linked residues accounted for approximately 4% of the polymer and are, therefore, quantitatively minor components of the polysaccharides. On the other hand, beech galactan is a polysaccharide with a major content of 6-linked galactosyl residues although the amount of the polysaccharide accounted for by the 6-linked residues had not been known well. The fact that 6-linked and 4-linked galactosyl residues are present in a single polymer has been established by the isolation of the trisaccharide: Gal (β1→6)-Gal (β1→4)-Gal.

### 1.3.4. Arabinogalactan

Arabinogalactans have been isolated from the tissues of a variety of dicots. However, no arabinogalactan has been isolated from a source known to contain only primary cell walls. Unlike the arabinans and galactans, there is considerable variation in the glycosyl compositions of the arabinogalactans (Darvill *et al.,* 1980). Arabinogalactans occur in two structurally different forms: arabinogalactans type I and arabinogalactans type II.

Arabinogalactan type I has a (1→4)-linked linear chain of β-D-galactopyranosyl residues with 20-40% α-L-arabinofuranosyl residues (1→5)-linked in short side chains connected in general to *O*-3 (McNeil *et al.,* 1984; Dey and Brinson, 1984). Pectins with arabinogalactans of type I attached are commonly found in food such as citrus (Labavitch *et al.,* 1976), potato (Jarvis *et al.,* 1981a; 1981b), soybeans (Aspinall *et al.,* 1967), lupin (Carré *et al.,* 1985), tobacco (Eda *et al.,* 1986), apples (Voragen *et al.,* 1986c; Schols *et al.,* 1990), onions (Ishii, 1982), kiwi fruit (Redgwell *et al.,* 1988), tomatoes (Seymour *et al.,* 1990), and cabbage (Stevens and selvendran, 1984). The structure of a type I arabinogalactan from soybeans (Aspinall and Cottrell, 1971) is shown in Figure 1.3. Their presence in walls is usually inferred from the presence of (1→4)-linked galactopyranosyl residues during methylation analyses of whole walls. Conformational energy calculations have led Bluhm and Sarko (1977) to conclude that linear (1→4)-β-D-galactan chains can adopt a double-helix structure.

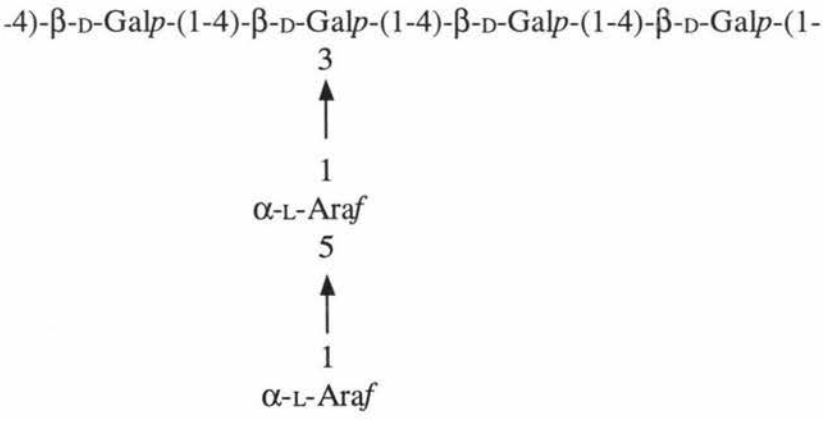


**Figure 1.3 Structure of a Type I Arabinogalactans from Soybeans**
**Gal*p*, Galactopyranose; Ara*f*, Arabinofuranose.**
**(From Aspinall and Cottrell, 1971)**

Type II arabinogalactan is a highly branched polysaccharide with ramified chains of β-D-galactopyranose residues joined by (1→3) and (1→6) linkages. The (1→3) linkages predominate in the interior chains, and the (1→6) linkages occur mainly in the exterior chains, which are generally terminated by L-arabinofuranosyl and to some extent by L-arabinopyranosyl residues (Aspinall, 1980; McNeil *et al.*, 1984; Clarke *et al.*, 1979). Pectins with type II arabinogalactans covalently attached have been found, for example, in apple (Aspinall and Fanous, 1984; De Vries *et al.*, 1983; Selvendran, 1985), rapeseed (Siddiqui and Wood, 1977; Larm *et al.*, 1976), lemon (Aspoinall and Cottrell, 1970), beet (Guillon and Thibault, 1989), and grape (Saulnier *et al.*, 1988). The type II arabinogalactans and arabinogalactan-proteins are water-soluble and are therefore likely to be extracted together with the pectic type I arabinogalactans. Type II arabinogalactans and arabinogalactan-proteins are thought not to be wall components but constituents of the extracellular space and possibly associated with the plasma membrane (Fincher *et al.*, 1983).

### 1.3.5. Arabinan

Arabinan polysaccharides have been isolated from the cell walls of many plant materials (Eriksson *et al.*, 1996), such as apples (Voragen *et al.*, 1987; Schols. *et al.*, 1990), sugar beet (Guillon and Thibault, 1989; Guillon *et al.*, 1989), suspension-cultured sycamore cell walls (Talmadge *et al.*, 1973), rapeseed hulls (Siddiqui and Wood, 1977), apricots (Siliha, 1985), carrots (Massiot *et al.*, 1988), cabbage (Stevens and Selvendran, 1984), onions (Ishii, 1982; Redgwell and Selvendran, 1986), pears (Bobsky and Schobinger, 1986), and pea hulls (Ralet *et al.*, 1993). However, most of these arabinans have contained small proportions of other glycosyl residues of uncertain significance and pure arabinans have been obtained only from mustard seeds (Rees and Richardson, 1966), the inner bark of *Rosa glauca* (Joseleau *et al.*, 1977), and a methylated primary cell wall polysaccharide fraction of suspension-cultured sycamore cells (Darvill *et al.*, 1980). The degree of polymerization of two arabinans from *Rosa Glauca* is 34 and 100 (Joseleau *et al.*, 1977). Methylation analysis of the primary walls of suspension-cultured pea cells suggested the presence of a similar polymer.

Arabinans are branched polysaccharides with a backbone of α-(1→5)-linked L-arabinofuranosyl residues, other α-L-arabinofuranosyl units being attached to varying numbers of residues at the *O*-2 and /or *O*-3 position (Voragen *et al.,* 1995). These may be generally represented by the partial structure shown in Figure 1.4. Several arabinans contain linkages additional to those shown in Figure 1.4, notably sidechains attached by α-(1→2)- L-arabinofuranosyl linkages to branch points in the backbone, which may or may not also, carry (1→3)-linked units. An alternative mode of attachment has been proposed by Stevens and Selvendran (1984), for the neutral arabinan from cabbage (*Brassica oleracea*) pectic polysaccharide fractions. From methylation studies, they postulate that the arabinan is attached to the acidic rhamnogalacturonan through the C (*O*) 4 of the (1→2)-linked rhamnopyranosyl residues via one to three (1→4)-linked β-D-galactopyranosyl residues. The structural investigation of these isolates has mainly involved methylation analysis and $^{13}C$ NMR spectroscopy.

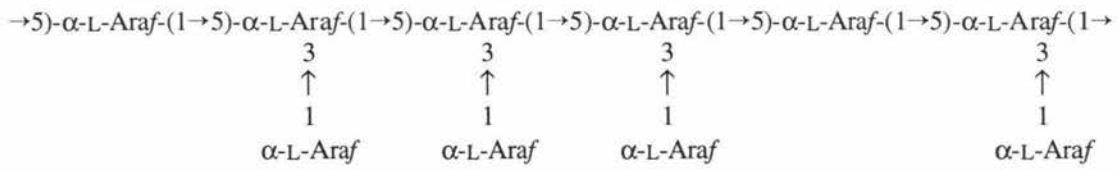


**Figure 1.4 General Structure of Higher Plant Arabinans.**
**Araf, Arabinofuranose.**
**(From Brinson and Dey, 1985)**

Arabinans are regarded as forming part of the neutral pectic fraction of the cell wall, and they have been isolated under strongly basic conditions, e.g., extraction with hot limewater. The so-called sugar beet arabinan is such a polysaccharide preparation (Hough and Powell, 1960; Hullar, 1965), but it is probably a degradation product in which other constituent sugars including galacturonic acid originate from the residual stubs of the rhamnogalacturonan chains of a pectin. Further evidence for the heteropolysaccharide nature of the sugar beet preparation was obtained by the isolation

of *O*-α-L-arabinofuranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→1)-glycerol from a Smith periodate degradation (Hullar, 1965).

The role of arabinans in seeds and plant cell walls is not known, but may be related to cohesion and bonding (Aspinall, 1973; Albersheim *et al.*, 1973; Kikuchi *et al.*, 1996). The linear arabinans can produce a spreadable gel that is similar to gelling maltodextrins (Kasapis *et al.*, 1992; Cros *et al.* 1994). Their rheological properties and resistance to human digestive enzymes suggest the possible use of α-(1→5)-arabinans as a fat substitute and they have been employed in ice cream and in chilled or frozen desserts (Cooper *et al.*, 1992). These polymers have also been used in the pharmaceutical and cosmetics industries (Cros *et al.*, 1994).

## 1.4. Protein

Proteins are quantitatively important components of the cell wall matrix. Primary walls may contain up to 10% protein (Bacic *et al.*, 1988). Some of them are only loosely bound and may be located in the periplasmic space (inner wall surface), whereas others are more tightly bound but can be eluted by agents such as 1 M NaCl, 3 M LiCl or 0.5 M $CaCl_2$ (Bacic, *et al.*, 1988). This suggests that they may be held in the wall by ionic forces. Others are not removed by these treatments and are often referred to as covalently bound, although nonextractability is not proof of covalent linkage. It is sometimes difficult to decide whether protein in wall preparations is true wall protein or adventitious cytoplasmic protein deposited during wall isolation (Harris, 1983).

There are several types of structural proteins in walls, but among these the family of hydroxyproline-rich glycoproteins (HRGPs, extensins) are the best-characterized class. Arabinogalactan-proteins (AGPs) are found in most higher plants and in many of their secretions (Fincher and Stone, 1983). A possible structure of a cell wall glycoprotein segment is given in Figure 1.5, taken from Clarke *et al.* (1979).

### 1.4.1. Arabinogalactan-protein

Arabinogalactan-proteins are a group of macromolecules characterized by a high proportion of carbohydrate in which galactose and arabinose are the predominant monosaccharides; there is also a low proportion of protein, typically containing high

levels of hydroxyproline (Fincher and Stone, 1983). In some situations, they are isolated as polysaccharides free from associated protein; in other situations, they occur in covalent association with protein, either refers to this group as proteoglycans, in which the protein component carries polysaccharide substituents (Gottschalk, 1972; Reid and Clamp, 1978) or as glycoproteins, in which the protein component is substituted by one or more oligosaccharide residues (Marshall, 1972).

Arabinogalactan-proteins containing arabinose and galactose have been isolated from aqueous extracts of plant material by the classical methods of salt and solvent precipitation, and by ion exchange chromatography (Churms, 1970) and affinity chromatography (Andrew and Stone, 1983), and precipitation by complexing with the β-glucosyl (Yariv) antigen, a specific (and diagnostic) method of recovering arabinogalactan-proteins from tissue extracts (Clarke *et al.,* 1979). Jermyn and Yeow (1975) isolated high MW polymers containing carbohydrate and protein, in the ratio approximately 8:1. They have been isolated from seeds representing all taxonomic groups of higher plants (Jermyn and Yeow, 1975), as well as from other plant tissues (Clarke *et al.,* 1978) and filtrates of cultured callus cells (Anderson *et al.,* 1977). In each case, the protein was remarkable for its high hydroxyproline content and the major monosaccharides were arabinose and galactose.

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, by alkaline degradation or by hydrazinolysis (Clarke *et al.,* 1979). Peptide bonds are cleaved during hydrazinolysis while the carbohydrate chains are resistant to such treatment. However, glycosyl-serine linkages are also vulnerable and serine is lost through β-elimination.

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 (Anderson *et al.,* 1977). The *Lolium multiflorum* arabinogalactan-protein has a MW of 200,000-300,000 and contains approximately 95% carbohydrate associated with 5% protein. The carbohydrate component of the glycoprotein is an arabinogalactan. The structure of the

arabinogalactan portion of this glycoprotein has been studied by methylation analysis and found structurally similar to a protein-free arabinogalactan, They consists almost entirely of galactose and arabinose in the ratio 1:1.7. 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 Figure 1.5 is only one of several possible interpretations of the analytical data.

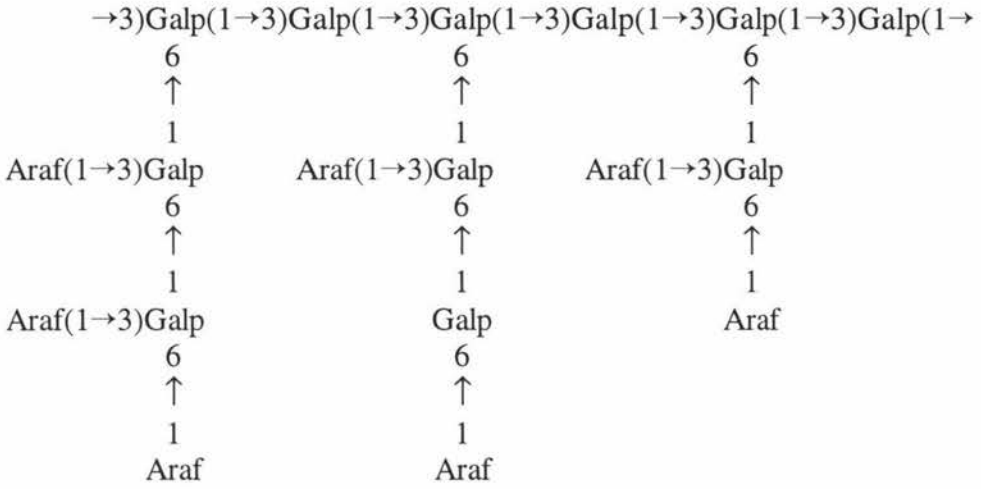


**Figure 1.5 Tentative Structure of Ryegrass (*Lolium Multiflorum*) Endosperm Arabinogalactan Protein** (Anderson *et al.,* 1977)

Possible functions of arabinogalactan-proteins can be inferred from their physical properties and their cellular localization. Arabinogalactan-proteins have potential for two major types of interaction: macromolecule-macromolecule or macromolecule-small ligand interactions (Fincher *et al.,* 1983). It seems likely that the arabinogalactan-proteins could interact with other polysaccharides such as pectins in the middle lamella, and it is possible that they may also make contact with membrane-bound and wall-associated lectins (Kauss, 1980). The function of arabinogalactan-proteins remains obscure. As they are so widely distributed and as their structure is so conserved, it seems unlikely that their function is trivial. As arabinogalactan-proteins are present at

the plasma membrane, they may be receptors of external signals such as the low molecular weight β-glucan elicitors of the phytoalexin response (Albersheim and Valent, 1978). Recently, attention has been focused on their structure and their potential role in growth and development of plants. Youl *et al* have presented evidence that two members of a major class of arabinogalactan-proteins, from styles of *Nicotiana alata* and cell suspension cultures of *Pyrus communis* respectively, undergo C-terminal processing involving glycosylphosphatidylinositol membrane anchors. An examination of the deduced amino acid sequences of other classical arabinogalactan-protein backbones shows that glycosylphosphatidylinositol-anchors may be a common feature of this class of arabinogalactan-proteins (Youl *et al*, 1998).

### 1.4.2. Hydroxyproline-rich Glycoprotein

Hydroxyproline-rich glycoproteins are important structural proteins in cell walls. They are characteristically found in dicotyledon primary walls (Roberts *et al.,* 1985; Wilson and Fry, 1986). Extensin levels increase in response to physiological stress and attack by pathogens. The name 'extensin' was adopted to convey a suggested involvement of the 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 Figure.1.6. The primary cell walls of dicots contain between 5 and 10% protein (Lamport, 1970; Preston, 1964; Talmadge *et al.,* 1973). The cell wall protein is exceptionally rich in hydroxyproline (20%). The wall protein also has a relatively high content of alanine, serine, and threonine. Fragments of the hydroxyproline-rich glycoprotein obtained from the primary cell walls of dicots invariably contain arabinosyl and galactosyl residues.

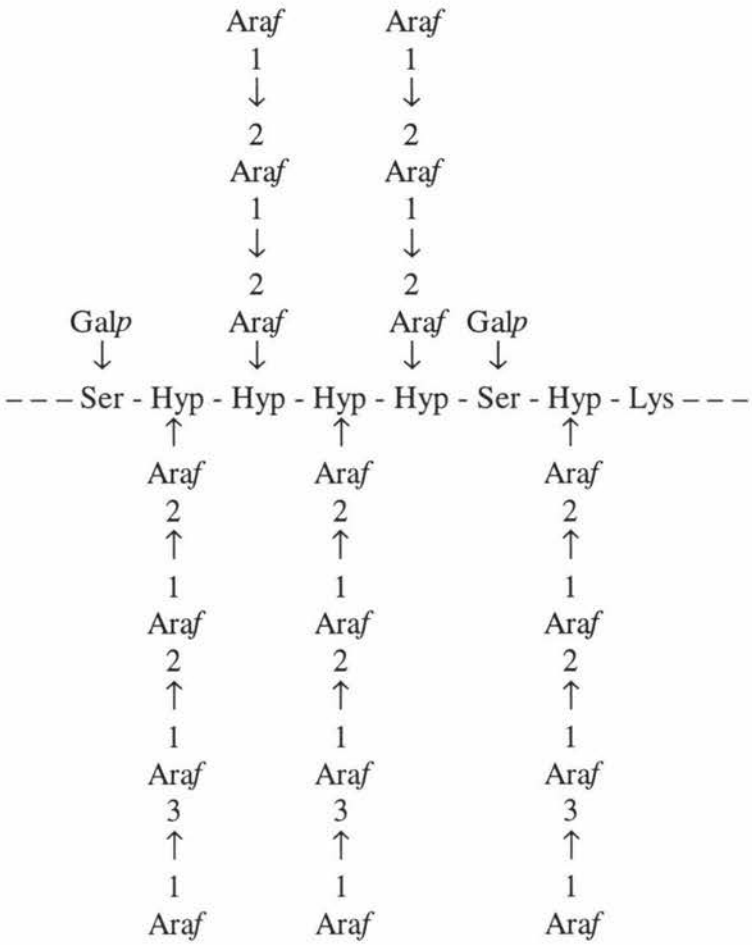


**Figure 1.6 Possible Structure of Cell Wall Glycoprotein Segment**

(From Clarke *et al.*, 1979)

In the glycoproteins of higher plant cell walls, carbohydrate is linked to protein through Ara-*O*-Hyp (Lamport, 1977) and Gal-*O*-Ser (Cho and Chrispeels, 1976; Lamport *et al.*, 1973). The carbohydrate consists of oligomers of three or four arabinofuranosyl residues. Hydroxyproline is usually glycosylated, although unsubstituted hydroxyproline residues occur in cell wall glycoproteins from some monocots (Lamport and Miller, 1971). The degree of glycosylation changes during development (Klis and Eeltink, 1979).

The hydroxyproline-rich glycoprotein fragments used for these studies have generally been isolated from the walls of suspension-cultured sycamore (*Acer pseudoplatanus*) and tomato (*Lycopersicon esculentum*) cells. A series of hydroxyproline-arabinosides have been isolated from such wall preparations (Darvill *et al.,* 1980). Extensins occur as extended rodlike molecules coated on the surface with covalently attached sugar residues. Chen and Varner (1985a, b) obtained cDNA and genomic clones for a wound-induced extensin from carrot and thus the full sequence of the 306-amino acid protein (molecular weight 34,000). There are no Gln, Asn, Asp, Trp, or Cys residues in the protein but it contains numerous repeat sequences, the most abundant of which is the pentapeptide (Ser-Hyp-Hyp-Hyp-Hyp).

The covalent attachment of arabinose and galactose to the hydroxyproline-rich glycoprotein of primary cell walls is a generally accepted fact (Lamport, 1970; Lamport *et al.,* 1973). However, the available evidence suggests that the hydroxyproline-rich glycoprotein is not covalently attached to any of the other cell wall polymers. The evidence does not rule out the possible existence of strong, noncovalent bonding between the hydroxyproline-rich glycoprotein and the other wall polymers. The hydroxyproline-rich glycoprotein is secreted by suspension-cultured sycamore cells into their culture medium (Keegstra *et al.,* 1973).

A composite model of a portion of the hydroxyproline-rich structural glycoprotein of dicot primary cell walls is depicted in Figure 1.6.

Hydroxyproline-rich glycoprotein has also been isolated from suspension-cultured monocot cells. The total amount of protein in the walls of suspension-cultured monocots and in the walls of maize coleoptiles is equal to or larger than the total amount of protein in the walls of suspension-cultured dicots (Burke *et al.,* 1974).

Several of the hydroxyproline-rich glycoproteins extracted from plant tissues have carbohydrate-binding activity; these glycoproteins have the characteristic of lectins. These lectins or lectin-like glycoproteins have components, which are similar to those of the cell wall hydroxyproline-rich glycoproteins (Darvill *et al.,* 1980).

## 1.5. Aims of the Thesis

Arabinans in plants are usually associated with pectins, as side chains on rhamnose residues, but their function is unknown. The arabinan is present not only in cell walls, but also as a soluble polysaccharide in the cells. Its abundance in the early stages of cell wall development is somewhat an enigma. Our laboratory has studied this area for a number of years. As a result of early studies, some preliminary structural information on the arabinans of *P. radiata* has been obtained. A highly branched arabinan has been found as part of the pectic fraction from *P. radiata* callus tissue (Andrew *et al.*, 1999), and preliminary work by Andrew and McKee (1997) has suggested the same type of compound to be present in vascular cambium of the same species.

The aim of this project is to isolate and purify an arabinan or arabinose-rich fraction from the cambium of *P. radiata* and to do some detailed analysis of its structure. This area would greatly facilitate understanding of the function of arabinans in plant cells and cell walls.

The arabinan from *P. radiata* cambium tissues was studied for the following reasons:

**a.** *P. radiata* is economically important to New Zealand both as a building material and to the pulp and paper industry and make a significant contribution to the export earnings. Any further knowledge gained on the polysaccharide composition of tissues in pine is advantageous because these polysaccharides relate to the growth and development of plants.

**b.** The arabinan content of *P. radiata* is relatively high and the presence of 3-linked and 3,5-branch residues is distinctive, but almost nothing is known of its function in any plants.

**c.** There is a lot of soluble pectin in cambium tissue and it is easy to isolate. Several neutral and acidic fractions have been identified in preliminary work. The neutral fractions are relatively enriched in arabinan. The acidic fractions are more typical of rhamnogalacturonan with arabinogalactan side-chains.

**d.** It should enable a comparison with results on other types of *P. radiata* tissues such as callus tissue.

**e.** The knowledge from this project will be useful to the further understanding of function of arabinans in plant cells and cell walls.