STRUCTURAL STUDIES OF A FUCOGALACTOXYLOGLUCAN FROM PINUS RADIATA PRIMARY CELL WALLS

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ABSTRACT

1. The changes in carbohydrate composition of elongating Pinus radiata primary cell walls were investigated. In the hemicellulose B extracts, a large increase in the percentage of non-starch, non-cellulosic, glucose was found to occur on cessation of cell-wall elongation.

2. By fractionation of the hemicellulose B extracts, with a variety of methods involving precipitation from an aqueous solution, a xyloglucan was purified. This xyloglucan was the major hemicellulose of the Pinus radiata hypocotyl cell wall.

3. Characterisation studies on the xyloglucan involved: quantitative analysis of the monosaccharides derived by nitric acid/urea hydrolysis; identification of the partial hydrolysis products derived by trifluoroacetic acid hydrolysis; quantitation of the sugar linkages using methylation by the Hakomori method; and analysis of the anomeric configuration of component sugars using chromium trioxide oxidation.

4. From the results a tentative structure has been suggested for the xyloglucan, consisting of a backbone of β-D-glucopyranose residues linked together by 1-4 glycosidic bonds, and with sidechains of single xylose residues linked through C-6 of the glucose units. Galacto and fuco-1,2-galacto sidechains are attached to some of the xylose residues, probably through the C-2 of the xylose.

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CHAPTER 1

INTRODUCTION

The cell wall of plant cells is an intriguing biochemical system that continues to defy definitive characterization. Peter Albersheim and his co-workers have recently completed the most comprehensive characterization of the composition of the wall of tissue cultured cells (Talmadge et al., 1973; Bauer et al., 1973; Keegstra et al., 1973). Nevertheless, the model for the structural arrangements of these polymers in the wall is still tentative and the exact roles of the various fractions not well understood (Albersheim, 1976). Moreover, information on the site and pathways of biosynthesis of wall polymers is very incomplete (Christpeels, 1976).

1.1 Ontogeny Of The Cell Wall

The origin of the cell wall can be traced to the appearance of the cell plate. This arises through fusion of vesicles (derived from dictyosomes and containing polysaccharides) deposited in the equatorial plane of the phragmoplast (Newcomb, 1969). These polysaccharides, shown to be rich in pectic substances (Northcote and Pickett-Heaps, 1966), become the building materials of the cell plate, which will contribute significantly to the middle lamella of the wall deposited by each of the daughter cells. Deposition of additional wall material follows on either side of the original plate as well as over the old mother cell wall.

The wall present during elongation growth is defined as the primary cell wall. The primary wall polymers will ultimately provide resistance in the horizontal direction to turgor pressure (the in vivo driving force for cell expansion) and thus contribute to the vertical elongation of the stem. Secondary wall polymers are deposited onto the inner primary wall. Rigidity is conferred upon the wall by the deposition of partially crystalline cellulose microfibrils at all stages of their development. The

fibrils are embedded in matrix materials imparting cohesion, strength and rigidity to an otherwise potentially fluid structure.

When plant cells grow in volume they are extraordinarily dynamic, and their wall components cannot be regarded as inert or subject only to passive re-orientation under pressure. Plant-cell expansion growth is irreversible and involves a net deposition of most wall materials, including cellulose, during growth. It is evident that both biosynthesis and turnover of wall components are crucial for shaping plant development.

To begin to understand cell wall metabolism during elongation, the structure of the wall itself must be known.

1.2 Cell Wall Constituents

The basic constituents of the cell walls of dicotyledons, monocotyledons and gymnosperms studied to date show remarkable conservation within groups and considerable overlap between groups (Burke et al., 1974). Figure 1 shows some of the more common sugars which occur as constituents of the polysaccharides. Major cell wall fractions have been described (Preston, 1974) and are listed below:

(1) Pectic substances (extracted by boiling in water for 12 hours or by hot ammonium oxalate)
(2) Hemicellulose (extracted usually by 4N KOH at room temperature)
(3) Cellulose (the residue of the above and often extracted with 72% sulphuric acid)
(4) Protein
(5) Lignin

Pectic substances, hemicelluloses and protein make up the major structural components of the primary cell wall matrix. Secondary walls have a greatly increased percentage of cellulose together with hemicellulose and lignin. Lignin is a characteristic component of secondarily thickened walls

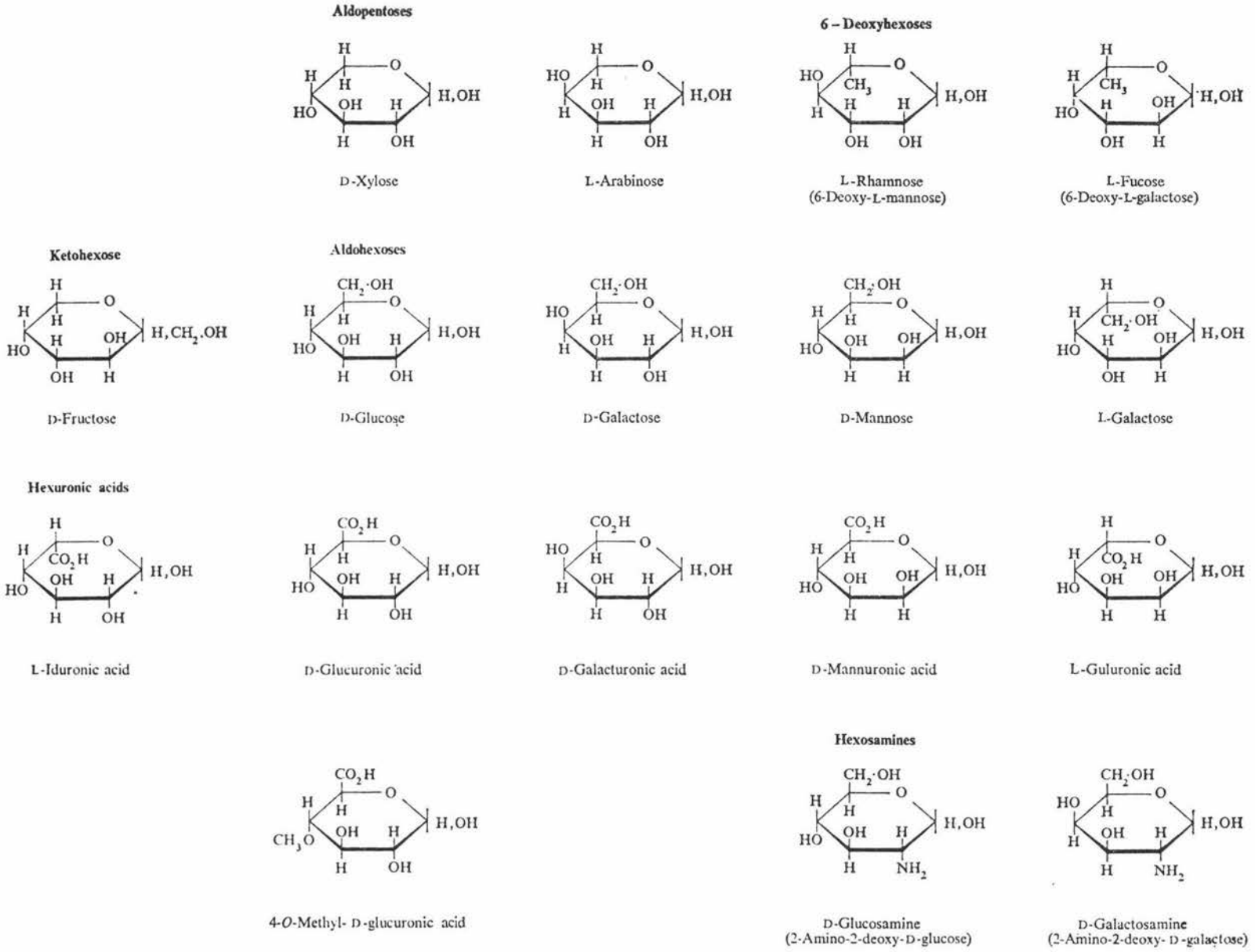


FIG. 1. Common sugar constituents of polysaccharides.

(Albersheim, 1965) and will only be described briefly.

Wilkie (1979) comments that the division implied by the variously defined terms hemicellulose, pectic substances, pentosans, linear and branched xylans, and others have limited scientific justification. They are useful laboratory terms insofar as they indicate starting-materials, procedures, preconceptions, and objectives in relation to studies of plantstuffs and their polysaccharides. Wilkie considers polysaccharides are best described by using chemical terms relating to their main structural features. However, much information is conveyed by the use of such terms as 'hemicellulose' to describe isolated fractions as it enables comparisons to be made between polysaccharides from various sources. These terms will be used in this work.

1.2.1 Cellulose

This is the major component of cell walls. Cellulose usually increases from about 20% of primary cell wall to 40% in mature walls. It is a simple linear polymer of β-1,4-linked glucopyranose residues. The glucose residues are present in the C1-chair conformation with the hydroxyl and hydroxymethyl groups all being in the more stable equatorial position. The degree of polymerisation of secondary-wall cellulose always remains constant and monodisperse in the cell walls of higher plants with a degree of polymerisation in wood between 13,000 - 16,000. The cellulose from primary cell walls however is of a much lower degree of polymerisation (2,000 - 6,000) and is more heterogeneous (Côté, 1977).

1.2.2 Lignin

Lignin is a three-dimensional polymer containing phenylpropane units linked together by C-O-C and C-C linkages. In softwoods, most phenylpropane units have one methoxyl group plus a phenolic oxygen. Lignin, a component

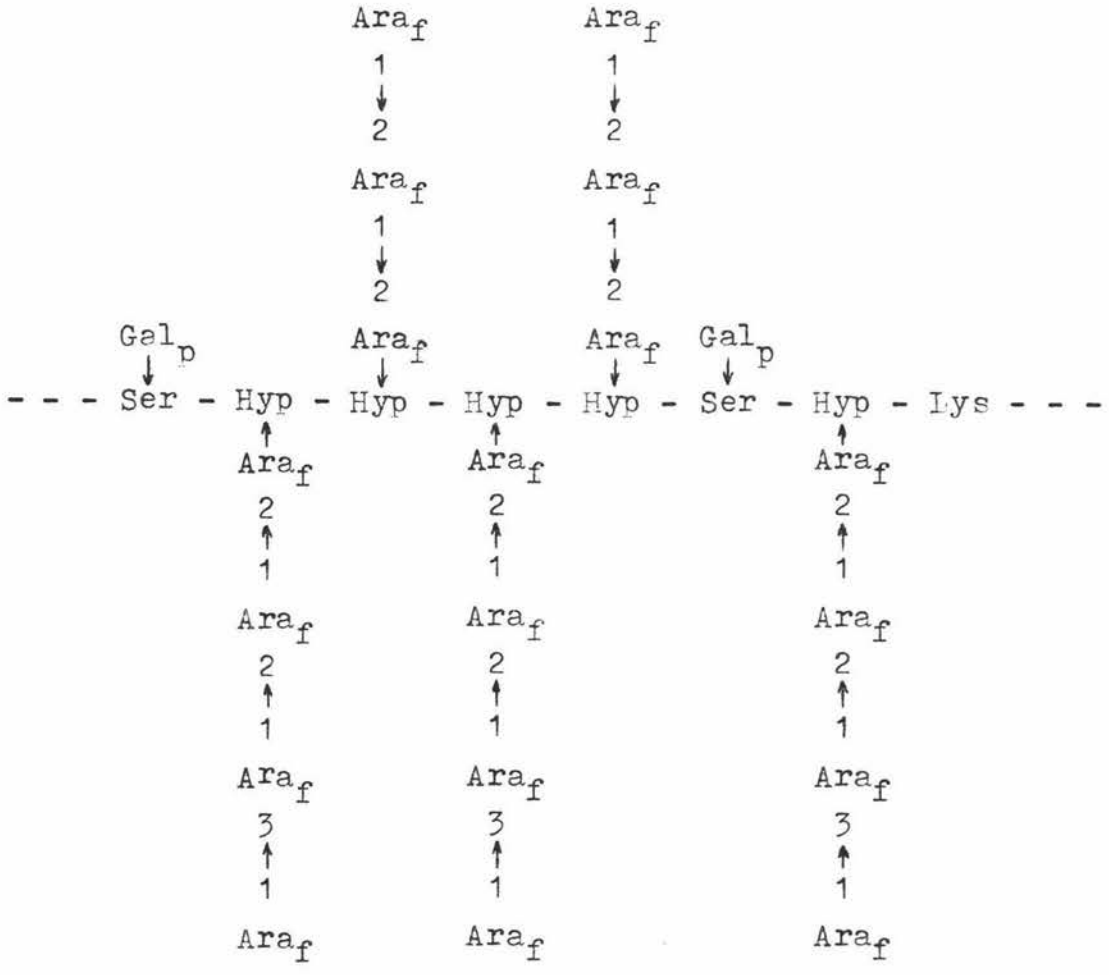
deposited with secondary wall thickening, increased from zero content in cambium cell walls (primary) of all plants, to about the same content as cellulose in mature softwood cell walls while in hardwood it reaches only half this amount (Côté, 1977). Lau et al. (1977) found lignin formation commences in developing Pinus elliotii hypocotyls only after cessation of cell wall elongation as would be expected. Evidence from several sources, such as the extraction of lignin-carbohydrate complexes, suggests that lignin is associated with or is combined with the matrix substances but not with the framework substance (Côté, 1977).

1.2.3 Protein

Protein is a quantitatively important component of the cell wall matrix (Lamport, 1965) although its role in cell wall growth is not firmly established. The cell wall protein is unusual in that about 30% of its residues are hydroxyproline (Lamport, 1970), an amino acid confined almost entirely to the cell wall in plants. Hydroxyproline is also a major constituent of animal connective tissue proteins, but is not glycosylated in these situations. In plant cell walls, L-arabinose oligosaccharides are attached O-glycosidically to most of the hydroxyproline (Lamport, 1967) and galactose by the same type of linkage to much of the serine (Lamport et al., 1973; Keegstra et al., 1973). A possible structure is given in Fig. 2.

The available evidence is in favour of the cell wall polysaccharides being connected to the hydroxyproline-containing protein through the serine residues of this protein. Due to the bonding of carbohydrate to fragments of the protein Lamport (1965) has gone as far as naming it extensin. This postulate, for control of cell wall extensibility, is supported indirectly by the finding that a decrease in growth rate is often associated with an increase in the level of cell wall hydroxyproline (Cleland and Karlsnes, 1967; Ridge and Osborne, 1970; Sadava et al., 1973).

Figure 2: Possible Structure Of Cell Wall Glycoprotein Segment (From Clarke et al., 1979).



1.2.4 Pectic Substances

The pectic polysaccharides of higher plants are a distinctive component of primary, but not secondary, wall material and are present also in the cell plate and middle lamella. Since cell-wall extension is restricted to primary growth, early theories on its mechanism laid special emphasis on a possible interrelation of wall plasticity and pectin metabolism (Stoddart and Northcote, 1967). However, cell-wall plasticity cannot be explained solely in terms of pectin metabolism, which has a specific role in new wall development.

Pectic substances are dissolved from the cell wall by aqueous solvents containing calcium chelating agents such as ethylenediaminetetraacetic acid (EDTA) or ammonium oxalate. They have been divided up by Aspinall (1973) into three polymer types.

(1) Neutral Arabinans

These are basically highly branched polysaccharides with predominantly α-L-arabinofuranose residues present. They contain α(1-5) and α(1-3) linkages (Fig. 3). They are usually in small amounts in most higher plants and their role in seeds and plant cell walls is not known. The arabinans have been isolated with arabinogalactan from lemon peel showing that the highly branched arabinans must be distinct species which cannot arise from partial degradation of the more complex neutral polysaccharide (Aspinall, 1973).

(2) Neutral Galactans and/or Arabinogalactans I (or II)

The galactans or arabinogalactans which are found in association with pectins are most commonly those based on linear chains of (1-4)-linked β-D-galactopyranose residues (Fig. 4). It is now apparent, however, that composition alone does not define structure since arabinogalactans of type II, similar in composition but of quite different structure to those of type I, have been found in association with pectins. Arabinogalactans of type II are most commonly found in coniferous woods and are particularly abundant in

Figure 3: General Structure Of An Arabinan.

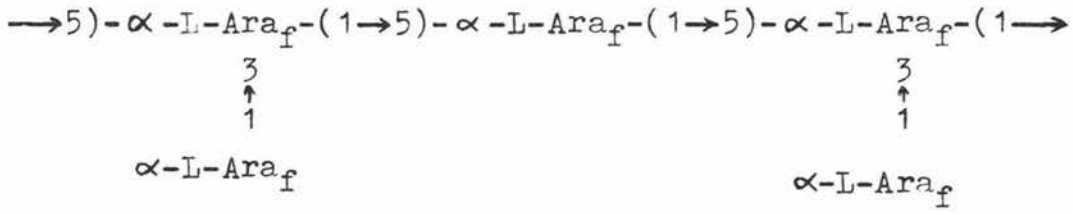
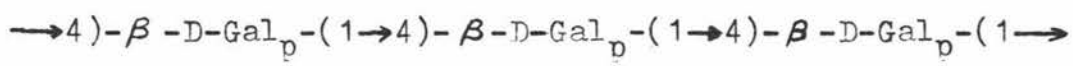


Figure 4: Structure Of Galactan.

Arabinogalactans of type I are also characterized by essentially linear chains of (1—4) linked chains of β-D-galactopyranose residues.



larches where the polysaccharide is present in the lumen of tracheids and ray cells rather than as a wall component. Arabinogalactans of this type contain highly branched structures in which β-D-galactopyranose residues are mutually joined by (1-3) and (1-6) linkages, the former predominantly in interior and the latter mainly in exterior chains. L-arabinofuranose, and to a smaller extent L-arabinopyranosyl residues, terminate some of the outer chains. The distribution of (1-6) linkages is largely as indicated in Fig. 5 but not necessarily in a completely non-ramified comb-like structure. Some arabinogalactans of this type also contain terminal units of D-glucuronic acid (or its 4-methyl ether).

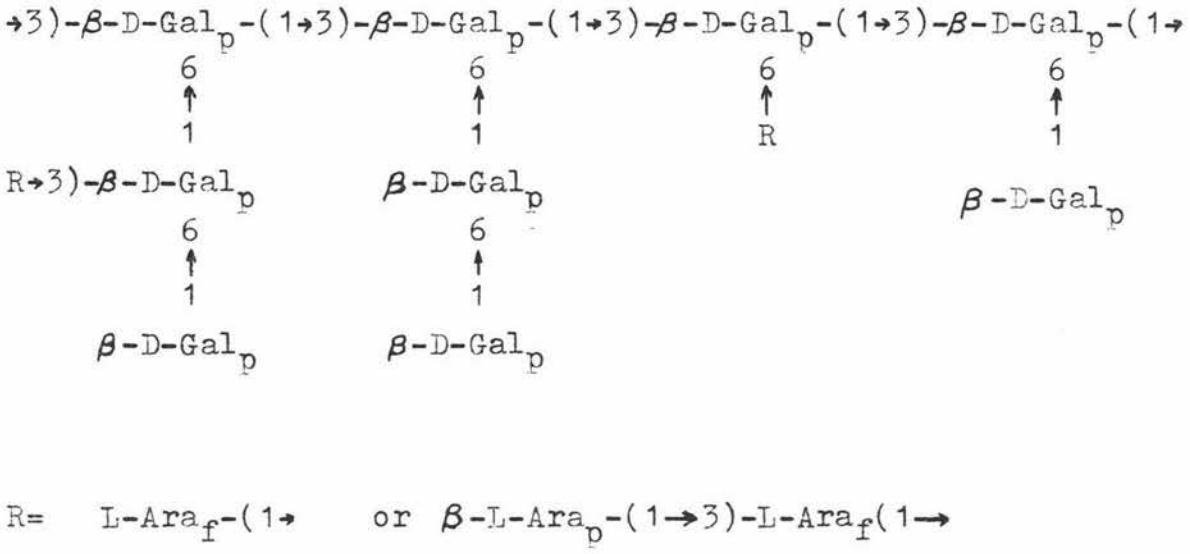
(3) Acidic Galacturonans or Galacturonorhamnans

Galacturonans are predominantly composed of linear chains of 4-linked α-D-galacturonic acid in the pyranose form (Fig. 6). In nature a large proportion of the galacturonic acid residues are methyl esterified. It is now apparent that pure galacturonans are of infrequent occurrence and that the majority of polysaccharides rich in galacturonic acid contain significant amounts of neutral sugar substituents. The 2-linked L-rhamnose occurs only in the interior chains, whereas residues of all other neutral sugars are encountered in the exterior chains only. Sidechains containing β-D-galactopyranose and L-arabinofuranose residues are the most characteristic (Fig. 6). Barrett and Northcote (1965) have shown that these sidechains in apple galacturonorhamnan are distributed irregularly in widely spaced blocks along the macromolecular chain.

Stoddart and Northcote (1967) have demonstrated a temporal linkage between the various pectic fractions of sycamore suspension-culture cell walls. Knee (1978) has produced evidence for a spacial arrangement in apple fruit cortical tissue, with a simple polymethylgalacturonate localised in the middle lamella, while a branched polymethylgalacturonate, with sidechains of arabinose and galactose residues, was concentrated in the primary wall.

Sidechains in acidic galacturonorhamnans of

Figure 5: Representative Structure For Arabinogalactans Of Type II.



R= L-Ara$_f$-(1→ or β-L-Ara$_p$-(1→3)-L-Ara$_f$(1→

Figure 6: Structure Of Galacturonan.

$\rightarrow$4)-α-D-Gal$_p$A-(1$\rightarrow$4)-α-D-Gal$_p$A-(1$\rightarrow$4)-α-D-Gal$_p$A-(1$\rightarrow$

General Structure Of A Galacturonorhamnan.

$\rightarrow$4)-α-D-Gal$_p$A-(1$\rightarrow$4)-α-D-Gal$_p$A-(1$\rightarrow$2)-L-Rha$_p$-(1$\rightarrow$

Ara$_f$, Gal$_p$, Xyl$_p$, Fuc$_p$.

D-xylopyranose residues alone or with appended D-galactopyranose or L-fucopyranose units have been found as important structural units in pectins from tissues with potential for rapid enlargement and/or rapid differentiation, such as pollen (Bouveng, 1965), cotyledons from soybeans (Aspinall et al., 1967) and white mustard seeds (Rees and Wight, 1969). In elongating cell walls, where a fluid matrix is required, it seems that pectic polysaccharides are high in rhamnose and side-chains and that these decrease in the pectic substances as the cell ages (Rees and Wight, 1969). Model building computations for galacturonans indicate that the insertion of the 2-linked L-rhamnose units interrupts the tendency to form ordered chain conformations (Rees and Wight, 1971). The rhamnose causes 'kinking' of otherwise regular chains forming a zig-zagged structure. This along with side-chains and de-esterification would prevent the pectic substances forming gels. This gelling ability depends on the formation of junction zones (Rees, 1969) where the polyuronide chains become aligned and form tightly ordered microcrystallites. It is highly possible that these physical attributes of galacturonorhamnans arising from its structure, play some role in the requirement for a fluid matrix in cell wall elongation.

1.2.5 Hemicelluloses

The term hemicellulose describes a rather indefinite group that has been variously defined. It usually designates polysaccharides of low molecular weight which normally occur in plant tissues together with cellulose, and which can be isolated from the original or delignified material by extraction with aqueous alkali. Most of the land plant hemicelluloses are heteroglycans which have a linear main backbone chain to which are attached short appendages of different types of sugars.

Most hemicelluloses have been isolated from mature cell walls. We can divide these hemicelluloses into two main types (Timell, 1964 and 1965):

(i) arabino-(4-O-methylglucurono) xylans(Fig. 7)
(ii) gluco- and galactoglucomannans(Fig. 8)

(i) The xylans make up 10 - 15% of mature softwood and 15 - 20% of hardwood cell-wall polymers. They are generally quite large molecules in gymnosperm with a degree of polymerisation of 100 - 120. 4-linked β-D-xylopyranose residues form the xylan backbone. Some of the β-D-xylose residues are substituted in the C-2 and/or C-3 positions by single unit side chains of 4-O-methyl-α-D-glucuronic acid and/or α-L-arabinofuranose residues respectively. The distribution of the acid side chains on the xylose residues is random (Rosell and Svensson, 1975). In gymnosperms the distribution of the α-L-arabinose residues along the xylan backbone is probably also random. Xylans in hardwoods generally lack arabinose, but, instead commonly bear O-acetyl groups at C-3 of the xylose residues (Timell, 1964).

(ii) Gluco- and galactoglucomannans constitute the bulk of the gymnosperm wood hemicellulose but are a minor constituent in angiosperms.

The water soluble galactoglucomannans are isolated in fairly low yields from softwoods. They are a homogeneous polymer with ratios of galactose : glucose : mannose of 1 : 1 : 3. β-D-glucose and β-D-mannose are linked (1-4) to form the backbone, while α-D-galactopyranose is linked to the C-6 position of both mannose and glucose residues. 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.

The alkali-soluble glucomannans are the main polysaccharides present in the cell wall of mature softwoods, accounting for approximately half the hemicellulose fraction of coniferous woods. These polysaccharides are structurally similar to cellulose and seem to be closely associated with the cellulose molecules in the cell wall. The mannose to

Figure 7: General Formula For Xylans.

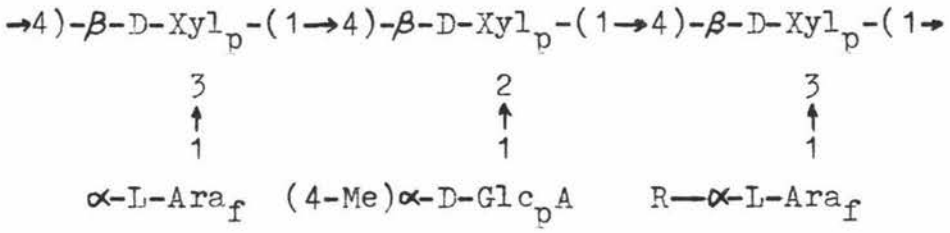
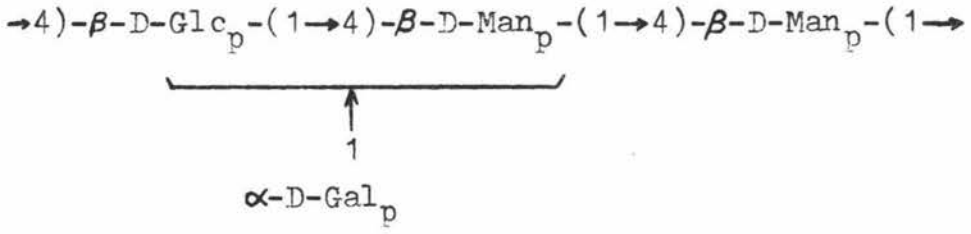


Figure 8: General Formula For Glucomannans.



glucose ratio does vary but in most softwood glucomannans it is about 3 : 1 compared with 2 : 1 in hardwoods. Also present is terminal D-galactose in quantities up to 5%. Softwood glucomannans are partially acetylated on either the C-2 or C-3 position of some of the mannose residues. These ester groups are lost under the alkaline conditions normally used for extraction of hemicelluloses.

Both the xylans and the glucomannans in mature cell walls are arranged in paracrystalline array between and in the same direction as the cellulose microfibrils and are strongly adsorbed (by hydrogen bonding) onto the microfibril surface. The linear structures of these polymers allow associations (as in the formation of microcrystallites) which have a uniting effect on wall polymers. They may therefore be important in interactions between the microfibrils and the cell wall matrix.

In some angiosperm seeds, the endosperm cell wall is greatly thickened by the deposition of galactomannans, glucomannans or mannans as a reserve polysaccharide. The galactomannans and mannans resemble the galactoglucomannans and glucomannans of wood, but lack the glucose residues.

1.2.6 Primary Cell Wall Hemicelluloses

Studies on primary cell walls have been hampered by having to deal with more than one type of primary cell wall and by the presence of secondary wall material. The use of young growing plants which contain predominantly a single cell type with little secondary thickening, together with the use of suspension-cultured cells, have however, enabled rapid progress in this area. Studies have shown the presence of a mixed-link β-1,3-β-1,4-glucan in monocotyledons and in at least one dicotyledon. Arabinoxylans have been demonstrated in monocotyledons with some results suggesting their presence also in dicotyledons. Xyloglucans have been found in dicotyledons.

Two of these polysaccharides, the β-glucan and the xyloglucan have been shown to turn over in elongating cell walls. This observation has led to their intensive investigation recently and is the primary reason for instigating this work.

1.2.6.1 Mixed-Link Glucan

The non-cellulosic mixed-linked β-D-glucan of monocots has been identified as a cell wall component of Zea, Hordeum, Sorghum, Triticum, Panicum, Arundinaria, Secale, Lolium and Avena (see Nevins et al. (1978) and refs therein cited). These polysaccharides are dissociated from the cell wall matrix of non-endospermic tissue by alkali. They have been characterised as linear homoglycans with both β-(1-3) and β-(1-4) glycosidic linkages. The mean ratio of (1-3) to (1-4) linkages was estimated at 3 : 7 (Stone, 1976). Nevins et al., (1978) using the very specific Bacillus subtilis and Rhizopus glucanases found that the cell wall β-D-glucans of five different grass species had 30.4 - 30.9% (1-3) β-D-glucosyl linkages in the molecules. These enzymes release both 3-O-β-cellobiosyl-D-glucose and 3-O-β-cellotriosyl-D-glucose with no significant disaccharides present, indicating that regions of repeating (1-3) glucosidic linkages represent at most only a small proportion of the total glucan complement.

There was at one time some argument as to whether the mixed-linked glucan was a cell wall constituent (Burke et al., 1974). Even though the glucan was found to be a component in vegetative tissues of various monocots (Buchala and Wilkie, 1973; Fraser and Wilkie, 1971) it has been postulated that this material is a storage product, not a structural wall component (Burke et al., 1974; Albersheim, 1976). In view of this criticism many workers have now given evidence for the β-D-glucan being a wall component and not a consequence of some molecular association induced during isolation (Nevins et al., 1977; Wada and Ray, 1978). It is possible that the analysis of six suspension-cultured monocots by Burke et al

(1974), which failed to reveal the presence of the glucan in all but one species, might be attributed to the use of a B. subtilis amylase preparation. This preparation is comtaminated by a β-D-glucanase capable of degrading only mixed-link glucans (Nevins et al., 1977). A similar mixed-link β-D-glucan has been reported from Phaseolus aureus - a dicot (see section 1.2.6.5).

1.2.6.2 Arabinoxylan

The other major hemicellulose present in monocot seedling primary cell walls is an acidic arabinoxylan (Darvill et al., 1978; Buchala, 1974; Wada and Ray, 1978). These arabinoxylans, like those of mature tissues already discussed, always contain a linear 1-4 linked xylan backbone. Frequent sidechains attached mainly at the 3-position (but also at the 2-position) comprise either: (a) single arabinofuranosyl residues; or (b) single 4-O-methyl glucuronosyl residues, (both (a) and (b) are common); or (c) arabinose, xylose and/or galactose oligosaccharides; or (d) glucuronic and galacturonic acid oligosaccharides. Similar arabinoxylans are found in several species of cultured monocot cells (Burke et al., 1974) and barley aleurone cells (McNeil et al., 1975). Some of these polysaccharides may also be acidic arabinoxylans although that from barley aleurone was reported to contain no uronosyl residues (McNeil et al., 1975).

The cereal arabinoxylans of both wheat endosperm (Mares and Stone, 1973) and barley aleurone cell wall (McNeil et al., 1975) can be separated into fractions with wide ranging arabinose to xylose ratios, indicating a highly irregular arrangement of the arabinosyl side groups. However, the Avena coleoptile glucuronoarabinoxylan yielded subfractions of similar arabinose : xylose ratio (Wada and Ray, 1978).

1.2.6.3 Xyloglucan

The major hemicellulose reported in dicotyledons is a xyloglucan. Kato and Matsuda (1976) have isolated xyloglucan from the hypocotyls of three leguminosae plants; Phaseolus aureus (mungbean), Glycine max and Vigna sesquipedalis. The P. aureus xyloglucan gave glucose, xylose, galactose and fucose in the approximate molar ratio of 10 : 7 : 2.5 : 1. A xyloglucan shown to turnover in Pisum sativum stem sections has been isolated and also found to have similar ratios.

The most thorough structural investigations have been done on the xyloglucans isolated from suspension-cultured cells. Suspension-cultured cells have been grown as an homogeneous tissue possessing primary, but no secondary, walls. These cells also secrete into their culture medium polysaccharides that appear to be structurally identical to the noncellulosic polysaccharides of the cell wall (Albersheim, 1976 and refs cited therein). The major isolated hemicellulose of Acer pseudoplatanus (Bauer et al., 1973), Phaseolus vulgaris (Wilder and Albersheim, 1973) and Rosa glauca (Barnoud et al., 1977) cell-suspension cultures is a fucogalactoxyloglucan of similar structure to that from Phaseolus aureus hypocotyl.

The constitutions of a wide range of dicotyledon cell walls have been examined by methylation analysis. Cell walls from tomato (Lycopersicon esculentum), soybean (Glycine max), Red Kidney bean (P. vulgaris) and sycamore cell-suspension cultures, as well as from 8-day-old Red Kidney bean hypocotyls, all gave gas chromatograms featuring as major peaks the components of a derivatised fucogalactoxyloglucan. Further, D. H. Northcote and coworkers have shown isolated sycamore cambial cell walls to have nearly identical composition to that of suspension-cultured cells and T. E. Timell and B. W. Simson found similar results for aspen cambial cell walls (quoted by Albersheim, 1976 p263). This information has convinced Albersheim and coworkers that such diverse dicot

plants as beans, tomatoes and sycamore trees, have architecturally very similar primary cell walls, containing a fucogalactoxyloglucan as a major hemicellulose.

Xyloglucan polymers have also been found in the seeds of many dicot plants. These have been designated 'amyloids' due to their blue staining property with iodine. Two subgroups are distinguished: (a) Fucogalactoxyloglucans (fucoamyloids) are found in mustard seed (Gould et al., 1971), rapeseed (Brassica) hull (Aspinall et al., 1977) and rapeseed meal (Siddiqui and Wood, 1977; Theander and Åman, 1978); (b) Galactoxyloglucans have been isolated from the seeds of Tamarindus indica (Kooiman, 1961; Srivastava and Singh, 1967), Tropaeolum majus (Hsu and Reeves, 1967; Aspinall et al., 1977), Annona muricata L. (Kooiman, 1967) and Sinapis alba (Gould et al., 1971).

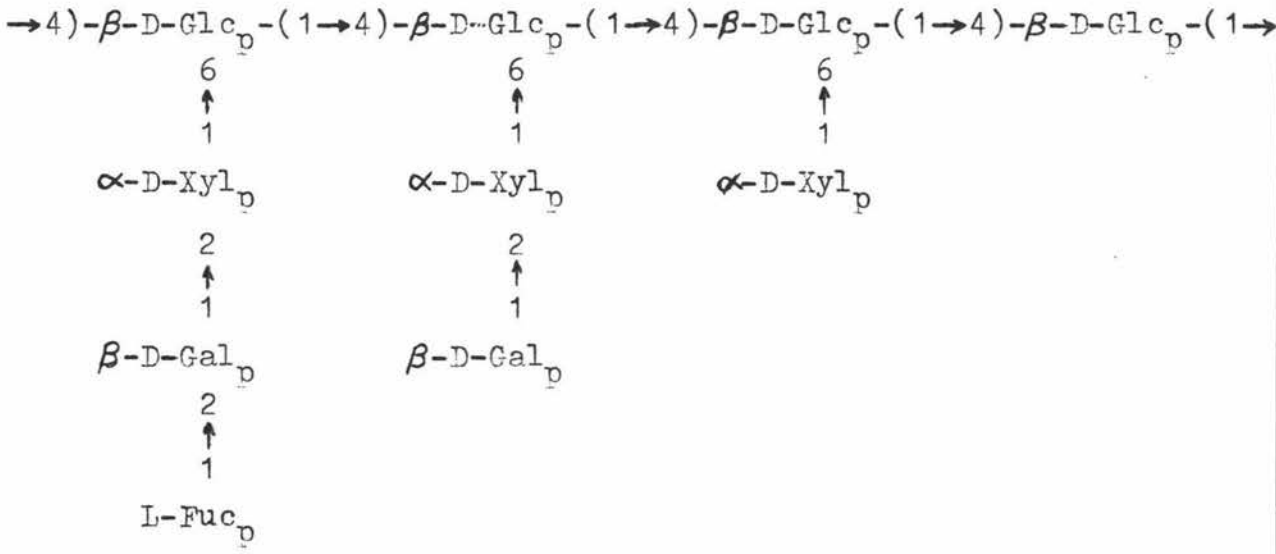
All the xyloglucan polymers isolated have a structure based on a repeating heptasaccharide unit which consists of four residues of β-(1-4)-linked glucose and three residues of terminal xylose linked to the 6-position of three of the glucosyl residues. Fucosyl-(1-2)-xylose and galactosyl-(1-2)-xylose side chains also are present in varying proportions in different species. A generalized structure for this polymer is shown in Fig. 9.

1.2.6.4 Callose

Another polysaccharide which seems to play a particularly dynamic role in cell development is callose, a linear β-1,3-glucan. Although not generally regarded as a hemicellulose, it is pertinent to consider it at this point. Callose is widely distributed, not only in the specialised plasmodesmata of sieve tube pores (Mc Nairn and Currier, 1968) but also in plasmodesmata generally. It has also been established as a polymer of the pollen cell wall eg. in Pinus mugo (Bouveng, 1963).

Most frequently, callose is classed as a wound-response material but experimental evidence also suggests it may play a role in regulating the intercellular movement of

Figure 9: General Structure Of A Fucogalactoxyloglucan.



substances in plant tissues. (McNairn and Currier, 1968). Its dynamism is documented by the cytological studies of Fulcher et al. (1975). Temporary depositions of this β-1,3-glucan are observed in cell plates of cells undergoing cytokinesis and in recently formed transverse cell walls of newly divided cells from coleoptile, primary leaf and emerging root tissues. There is little or no evidence of callose in ungerminated embryos. This suggests that a callose-like substance may be associated with cell wall formation and (or) expansion but the role and fate of the polysaccharide are unknown.

1.2.6.5 Angiosperm Primary Cell Wall

The overall impression emerging is that the matrix material of monocot and dicot primary walls is rather different. However, the generalisation that the co-occurrence of the mixed-link glucan and arabinoxylan is a distinctive characteristic of monocot primary cell walls while the xyloglucan is a component of dicot walls, must be qualified by certain facts and observations.

A water-soluble mixed-link β-D-glucan has been reported to occur transiently in the hypocotyls of Phaseolus aureus, a dicot (Buchala and Franz, 1974). This glucan has (1-3) and (1-4) linked D-glucopyranosyl residues in the molar ratio of 1.0 : 1.7.

An arabinoxyloglucan has been isolated from rice (Oryza sativa) endosperm cell walls (Shibuya and Misaki, 1978). This monocot tissue also contains the expected mixed-link β-glucan and arabinoxylan. Labavitch and Ray (1978) reported the fractionation of a xyloglucan from the coleoptile of Avena, a monocot. Enzymatic degradation gave a pentasaccharide and a trisaccharide that contain xylose and glucose in the linkages typical of xyloglucans.

Although no arabinoxylans have been isolated from dicot primary cell walls there are results suggesting a minor presence. An extracellular acidic 1,4-linked xylan was

isolated from sycamore cell-suspension culture (Keegstra et al., 1973) while fractions containing a high percentage of xylose were extracted from lupin hypocotyls (Monro et al., 1976).

It is possible that with detailed analysis of primary cell walls, the three hemicellulose types will be found present in a wide range of species but with a considerable variation in ratios between species. For example, the cell walls of the starchy endosperm of barley consist of an amorphous ground substance approximately 75% mixed-link β-glucan and 25% arabinoxylan (Fincher, 1975). In contrast, the principal classes of polymers present in the Avena coleoptile wall matrix, glucuronoarabinoxylan and mixed-link glucan, comprise approximately 55% and 30% of the hemicellulose fraction respectively, with xyloglucan a minor component (Labavitch and Ray, 1978).

1.3 Cell Wall Models

The nature of the bonds between the different polysaccharide fractions of cell walls is not clearly understood and the relationship between fractions is therefore not clear. Preston (1974) believes that the majority of bonds are of three types: (a) hydrogen bonds; (b) salt bridges; and (c) Van der Waals forces, with few if any covalent bonds, while Keegstra et al. (1973) postulated that covalent bonds were most common, and they put little emphasis on other linkages except for hydrogen bonding between xyloglucan and cellulose. From the foregoing it is obvious that the fine structure of the wall is controversial. A protein-glycan network, in general, is now supported by the studies of Albersheim and colleagues (Bauer et al., 1973; Keegstra et al., 1973; Talmadge et al., 1973). A potentially more specific dissection of the plant cell wall has been initiated by these workers, who have used enzymes in combination with chemical treatment to digest wall components and from this to deduce the nature of the interaction between the various subfractions.

In the emerging picture of the dicotyledonous cell wall, the cellulose microfibrils are coated with a single layer of xyloglucan by hydrogen bonding. The xyloglucan of one fibril may be linked to the xyloglucan of another fibril by covalent cross-linking through pectic polysaccharides Fig. 10. It is however doubtful that covalent linkages are the only linkage type between matrix polymers (Monro et al., 1976). A similar arrangement has been proposed for the walls of monocotyledonous plants, except that xyloglucan backbone is replaced by an arabinoxylan backbone (Burke et al., 1974). The picture for monocot structure is complicated by the presence of a mixed-linked β-D-glucan which undergoes rapid turnover during cell wall elongation (Huber and Nevins, 1979).

It should be emphasized that the above are simple models of the cell wall based upon analyses of very few cell types, and it seems likely that further analysis will complicate the picture.

1.4 Cell Wall Elongation

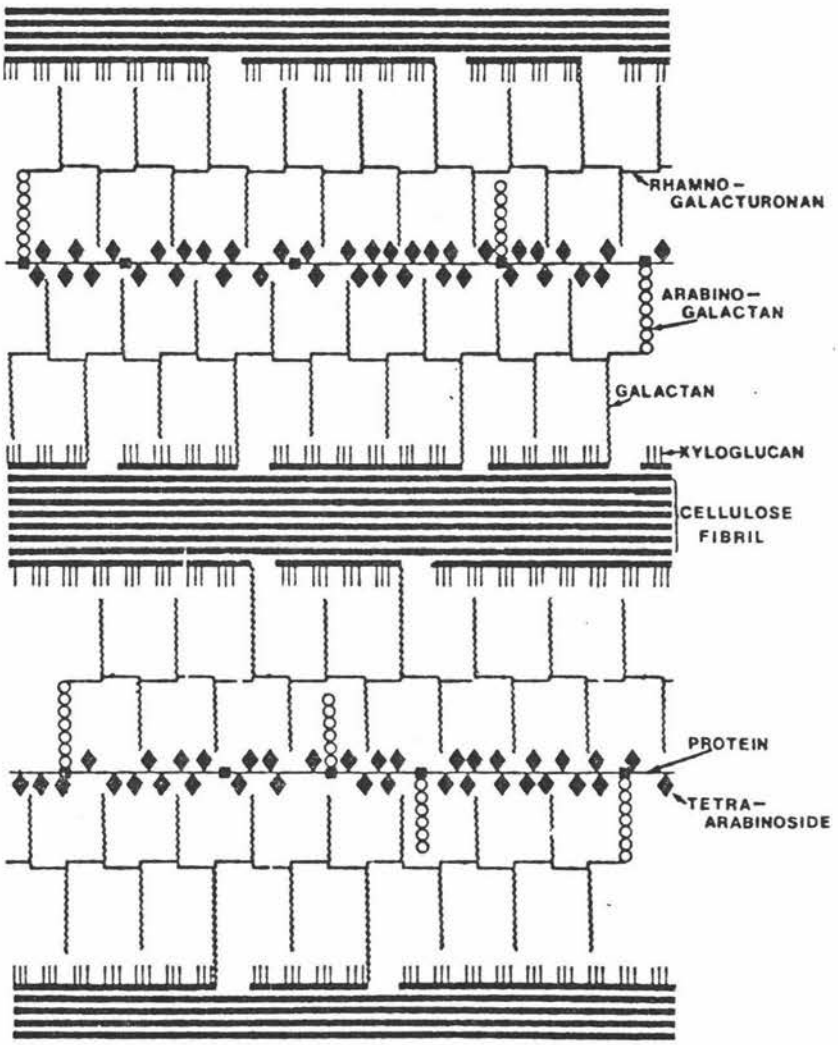
The biochemically dynamic nature of the primary cell wall has been recognised for some time (Lamport, 1970), both synthesis and breakdown of polysaccharides occurring together and leading to wall turnover. These processes are known to be strikingly increased by indole acetic acid (Lamport, 1970). In general terms, the mode of cell wall elongation in response to auxin has been studied from three major points of view: physical changes in the wall; enzymatic or non-enzymatic wall loosening; and turnover of wall components with synthesis of new wall material.

1.4.1 Physical Changes

Cleland (1971) has reviewed the considerable progress made in the study of cell wall physical changes where cell extensibility is divided into components such as plasticity, elasticity and 'creep'. He has pointed out the limitations of the physical measurements and the uncertainty which

FIGURE 10: Model of Sycamore Primary Cell Wall

(From Albersheim, 1976)



surrounds the nature of the properties being measured. Despite this, extension which is promoted by auxin and low pH can be correlated at least in part, with the measured changes in the physical characteristics of the wall.

1.4.2 Enzymatic Or Non-enzymatic Wall Loosening

A biphasic growth response has been demonstrated to occur following auxin application (Kohler, 1956) and this has since been confirmed in many tissues. The rapid effect of auxin on cell elongation is considered to be mediated by wall loosening, new wall synthesis and increased turgor pressure (Cleland, 1971). There is considerable evidence that hydrogen ions also catalyze the relaxation of the wall in a manner similar to that catalyzed by hormones (Adams et al., 1973; Evans, 1967; Rayle, 1973). There is also evidence that the hormones activate ion pumps within the cell membrane and that these ion pumps lower the pH of the wall (Cleland, 1971, 1973; Fisher and Albersheim, 1974). Auxin addition produces such a rapid response that de novo protein synthesis and de novo polysaccharide synthesis cannot participate in this initiation. An hypothesis based on these considerations would suggest that the direct action of the hormones is on the cell membrane, and that the reactions within the cell wall, which permit elongation growth, take place more efficiently at pH 5 than at pH 7. No bonds within the cell wall which would be non-enzymically degraded at pH 5 but which are stable at pH 7 have yet been found. However, Rayle and Cleland (1972) have shown that the cell walls of freeze-thawed coleoptile sections are weaker when buffered at pH 5 than at pH 7 only if the coleoptile sections have not been subjected to treatments that would denature enzymes. This supports the idea that the wall-loosening process is mediated by enzymes that remain active after freezing. A critical catalytic function for wall enzymes in plants is supported by evidence that wall enzymes play such a role in growth of bacterial cells (Fiedler and Glaser, 1973).

Serious limitations have, however, been found in the interpretations of most studies on enzyme involvement in wall extension. For example, exogenous application of enzyme mixtures containing β-1,3-glucanase and cellulase which was shown to rapidly stimulate elongation of segments of Avena coleoptile (Masuda and Wada, 1967) or Pisum epicotyl (Wada et al., 1968) could not be repeated by other workers (Cleland, 1968; Ruesink, 1969; Nevins, 1970). Also, although Datko and Maclachlan (1968) correlated auxin-induced swelling with the promotion of cellulase activity in pea epicotyls, Ridge and Osborne (1969) failed to observe a similar promotion under the same conditions. Thus results give conflicting evidence. As well, such observations are difficult to interpret unless the precise location and substrate specificity of the enzyme is known.

1.4.3 Turnover Of Wall Components And Synthesis Of New Wall Material

Turnover of various wall components is a possible mechanism whereby bond breakage and therefore wall loosening might be presumed to occur. Ray (1969) reviewed the then existing data and concluded that the early turnover studies were not interpretable for technical reasons related to the presence of starch, differential solubility of various fractions and the ill-defined nature of the wall components.

In a recent study of the effect of auxin on turnover of wall polysaccharides in pea stem segments which had been prelabelled with ^{14}C-glucose, Labavitch and Ray (1974a) noted that there was no effect of the hormone on turnover of the majority of the wall carbohydrates, but they did note changes in a pectinase-degradable xyloglucan. They found that auxin induced the conversion of an insoluble wall xyloglucan into a water-soluble form. The release of the xyloglucan was measurable within 15 min of hormone treatment, and was dependent upon cellular metabolism but independent of elongation (Labavitch and Ray, 1974b).

Subsequently Jacobs and Ray (1975 and 1976) showed that acidification could mimic the auxin effect and cause the release of a water-soluble xyloglucan from cell walls of Pisum. Labavitch has since demonstrated that the xyloglucan turned over in peas is in fact like that reported in beans and sycamore (quoted Albersheim, 1976 p270). Gilkes and Hall (1977), using gravmetric and pulse chase studies, demonstrated a turnover of polysaccharides in pea epicotyl cell walls. Their studies also suggest that auxin promotes the turnover most notably of a xyloglucan.

With monocots, auxin-induced growth of seedling sections is accompanied by a significant decrease in a non-cellulosic glucan component of the cell wall. This has led to suggestions that the glucan is involved in extension growth. Evidence consistent with the idea that glucan metabolism serves some role in this process is shown by the fact that mannitol restricts extension but not glucan metabolism (Loescher and Nevins, 1973). The results of Masuda and Satomur (1970) likewise indicate that glucan metabolism is involved in auxin-induced extension, since certain Sclerotinia glucanase preparations appear to mimic, in part, the action of auxin.

An examination of the β-glucans from Avena sativa, Triticum vulgare and Hordeum vulgare showed that with increasing plant maturity extended turnover occurs. There was a fall in the value of the ratio of β(1-3) to β(1-4) linkages, a decrease in the average molecular weight and, on a weight basis, a decrease in the amount of β-glucan present in the plant tissues (Buchala and Wilkie, 1973 and refs therein cited).

In order to avoid the removal of hydrolases that are only loosely associated with walls, glycerol has been used as a nonaqueous medium for cell wall isolation. Indeed, when incubating cell wall fragments of corn coleoptiles isolated in glycerol the original weight is considerably reduced by autolysis; the analysis of the solubilized polysaccharide has shown that at least 90% of this activity was attributed to the release of a mixed-link β-D-glucan

which constitutes as much as 11% of the cell wall (Kivilaan et al., 1971; Huber and Nevins, 1979). This autolysis reaction mimics processes which accompany auxin-induced elongation.

These findings implicate the turnover of a β-1,3-β-1,4-glucan in monocots. As well, Franz (1972) demonstrated hypocotyls of Phaseolus aureus underwent dramatic decreases in non-cellulosic glucose contents. This decline was attributed to the loss of a water-soluble mixed-link glucan (Buchala and Franz, 1974) which was absent from the water-soluble fraction of older hypocotyls. However, this decrease could also be due to the loss of a xyloglucan shown to be present in the alkali extract of P. aureus hypocotyls (Kato and Matsuda, 1976).

It thus appears, that specific wall components such as this mixed-link β-D-glucan, may impart structural rigidity to the primary cell wall. The lysis of these polysaccharides which takes place during cell elongation may be responsible for the necessary wall plasticity. In pea epicotyl segments, wall synthesizing enzymes undergo an increase in activity with auxin addition eg. β-glucan synthetase undergoes a two-fold increase. To reconcile these results with the release of soluble xyloglucan fractions from auxin-stimulated pea epicotyls (Labavitch and Ray, 1974) one can propose that wall elongation proceeds by a "break and repair" mechanism. Breaks might be induced by enzymatic or chemical means with a concomitant release of chopped out sections. Turgor would cause separation between breaks and these could be repaired enzymatically.

It is also possible that cellulose deposition may undergo a similar process. Maclachlan (1977) points to the fact that electron micrographs show fibril terminals readily visible in walls of algae and bacteria but absent in plants. Their absence raises the possibility that microfibrils in growing multicellular tissues extend via an insertion mechanism, wherby precursors are incorporated in such a way that breaks never appear. Several observations agree with such a "cellulose synthetase complex" including the presence of

potent cellulase that have been identified bound to the inner wall in growing regions of many higher plants.

1.5 Outline Of Present Investigation

The foregoing discussion on primary cell wall structure and elongation growth relates almost exclusively to angiosperms. Although the structures of gymnosperm wood polysaccharides have been fairly extensively studied, the studies of primary cell walls of gymnosperms have been relatively meagre. Thornber and Northcote (1961a,b) studied the monosaccharide composition of polysaccharide fractions from Pinus ponderosa cambium and Burke et al. (1974) briefly examined the linkage patterns of the sugar residues in cell walls of Pseudotsuga menziesii suspension-cultured cells. There appear to be no detailed structural studies of primary cell wall polysaccharides in any gymnosperm.

The growth and vegetative propagation of Pinus radiata is being investigated in several laboratories (see eg. N.Z. Forest Research Institute Annual Report 1977), but in order to understsnd the biochemical basis of growth in Pinus radiata it is necessary to understand something of the structure and turnover of the primary cell wall. With this in mind, an investigation was initiated on the structure and turnover of polysaccharides in the growing hypocotyl of Pinus radiata seedlings.

Since work with angiosperms has implicated a role, in the cell elongation process, for turnover of either a mixed-link β-glucan or a xyloglucan, a search for a similar polymer in the elongating hypocotyl of Pinus radiata was undertaken. The presence of a possible xyloglucan had been reported in the bark of Picea engelmanni (Ramalingham and Timell, 1964) and red spruce compression wood (Schreuder et al., 1966). Typical xyloglucan oligosaccharides were obtained on enzymic hydrolysis of a glucomannan from Pinus banksiana wood (Perila and Bishop, 1961). In addition, an acidic β-glucan, laricinan, with 1-3 and 1-4 linkages in a linear backbone, was reported in cell walls of compression

wood of Larix laricina and in Pinus resinosa xylem ray parenchyma cell walls. Laricinan was reported to have a ratio of 1-3 to 1-4 linkages of 13 : 1 (Hoffmann and Timell, 1972) which differed from the mixed-link β-glucans of monocots and Phaseolus aureus. Other non-cellulosic glucans reported from Pinus tissues include callose (Pinus sylvestris phloem, Fu et al., 1972; Pinus mugo pollen, Bouveng, 1963) and starch. However, no evidence is available in the literature on the occurrence of xyloglucans or mixed-link glucans or any other rapidly metabolising polysaccharides in elongating gymnosperm cell walls.

Consequently the project fell into two natural categories. The initial phase was to identify a glucan-type polymer showing changes in absolute levels during elongation growth of Pinus radiata hypocotyls. It was therefore necessary to monitor the proportions of glucose and other sugar residues in polysaccharide fractions from hypocotyls at different stages of growth, and to distinguish the glucose-containing component from starch and cellulose.

The second phase of the work was a structural characterisation of the glucan found in the first phase. For this, methylation analysis, partial hydrolysis and chromium-trioxide oxidation were all employed in determining sugar linkages and anomeric configurations.