Chemical Characterisation of Compression Wood in Plantation Grown *Pinus radiata*

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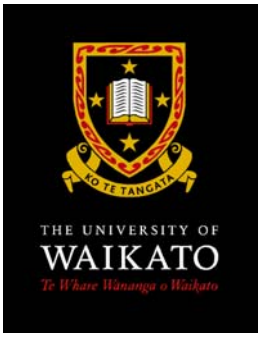

Abstract

The primary objective of this study was to find out if changes in chemistry could be used to quantify *Pinus radiata* compression wood severity or degree of compression wood development. Basic chemical composition and the lignin structure was assessed for a range of different compression wood samples sourced from juvenile wood, mature wood, earlywood, latewood, branches, knots, 2-year and 1-year old *Pinus radiata*. Fluorescence microscopy was used as the reference method to assess the degree of compression wood development. Lignin structure of compression wood was studied by thioacidolysis, size exclusion chromatography, and thioacidolysis/^{31}P NMR spectroscopy.

Variation in the basic chemical composition and lignin structure with compression wood severity was ascertained. Results showed that, as the severity of compression wood changed, progressively from normal through mild to severe, all chemical parameters commonly associated with compression wood changed concurrently. With increasing severity lignin and galactose levels increased while glucose and mannose levels decreased.

Lignin structural changes were also associated with changing severity of compression wood. Levels of *p*-hydroxyphenyl (H) releasable β-ethers increased and guaiacyl (G) releasable β-ethers decreased. Similarly, levels of uncondensed *p*-hydroxyphenyl units increased, while uncondensed guaiacyl units decreased. Similar proportions of condensed guaiacyl units were present in compression wood and normal wood. Similar trends in chemical composition were observed between the compression wood and related opposite wood in branches, knots and young wood of *Pinus radiata*.

A number of chemical parameters changed linearly with compression wood severity. They were: the amount of lignin and galactose, the galactose/glucose ratio and *p*-hydroxyphenyl content in lignin. Parameters based on the *p*-hydroxyphenyl unit content in lignin, the H/G releasable β-ether ratio, releasable *p*-hydroxyphenyl β-ether units and uncondensed *p*-hydroxyphenyl C9 units are most suitable indicators of compression wood severity as they spanned a larger range relative to the normal wood levels and were not influenced by the morphological origin of wood samples.

Chemical methods for quantifying compression wood severity should focus on the detection and measurement of these parameters.

Galactan present in *Pinus radiata* compression wood was isolated and characterised. Structural investigation by methylation analysis and NMR spectroscopy revealed that this galactan was largely composed of (1→4)-linked β-D-galactopyranose residues. No evidence was found to indicate the presence of any branches.

Characterisation of lignin in cell wall fractions of *Pinus radiata* normal wood revealed that middle lamella lignin has a higher lignin content, a lower amount of releasable β-ethers and a more condensed lignin than the secondary wall lignin. Levels of releasable *p*-hydroxyphenyl units were not higher in middle lamella lignin.

A new method based on thioacidolysis and ^{31}P quantitative NMR spectroscopy for estimation of the degree of lignin condensation of the phenolic and etherified C9 units in *in situ* wood lignin is described. Using this method it was found that phenolic C9 units in *in situ* lignin were considerably less condensed than etherified C9 units in both compression wood and normal wood.

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List of Abbreviations

%	percent
δ	chemical shift
µL	microlitres
µm	micrometres
µmol	micromoles
µs	microseconds
Ara	arabinose
Å	angstrom
AMU	atomic mass units
cm	centimetre
CPMAS	cross polarisation magic angle spinning
CSF	canadian standard freeness
CW	compression wood
DEAE	2-(diethylamino) ethyl
DEPT	distortionless enforcement by polarisation transfer
DFRC	derivatisation followed by reductive cleavage
DHP	dehydrogenation polymer
DMSO	dimethylsulphoxide
DP	degree of polymerisation
DRIFT	diffuse reflectance infrared fourier transform
eV	electron volt
EW	earlywood
GC	gas chromatography
g	gram
G	guaiacyl
Gal	galactose
h	hours
H	*p*-hydroxyphenyl
HP	Hewlett Packard
Hz	hertz
i.d.	internal diameter
ISTD	internal standard

IR	infrared
J	coupling constant
kDa	kilodalton
kPa	kilopascal
kW	kilowatt
kWh/o.d.t	kilowatt hours/oven dry tonne
L	litre
LCC	lignin carbohydrate complexes
LSD	least significance difference
LW	latewood
m	metre
mm	millimetres
Man	mannose
MCW	mild compression wood
MFA	micro fibril angle
mg	milligram
MHz	megahertz
min	minute
mL	millilitres
MS	mass spectroscopy
MSD	mass selective detector
MWL	milled wood lignin
No.	number
NMR	nuclear magnetic resonance
NW	normal wood
OW	opposite wood
ppm	parts per million
psi	pounds per square inch
PMAA	partially methylated alditol acetates
PTFE	polytetrafluoroethylene
py	pyrolysis
rpm	revolutions per minute
s	seconds
S2	secondary cell wall
S2L	outer secondary cell wall layer

S3	tertiary cell wall
SCW	severe compression wood
SEC	size exclusion chromatography
SEM	scanning electron microscopy
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMP	thermomechanical
TMS	trimethylsilyl
UV	ultraviolet
Wt	weight
Xyl	xylose

CHAPTER 1

1 Thesis Overview

This chapter introduces the topic of compression wood, and outlines the purpose of the investigation and thesis organisation. A detailed review of compression wood is given in Chapter 2.

1.1 Compression wood

If a tree is displaced from its vertical position by external influences such as wind, snow or slope, a special tissue, known as reaction wood is formed on the lower side of the stem or branch. In gymnosperms (softwoods) this tissue, usually called compression wood, exerts a compressive stress that serves to push the stem upright (*1*,*2*). Formation of compression wood (CW) provides trees with a mechanism by which stems are reoriented after displacement from the vertical. It results in a degree of elliptical growth and an off-centre pith in stems (Figure 1.1).

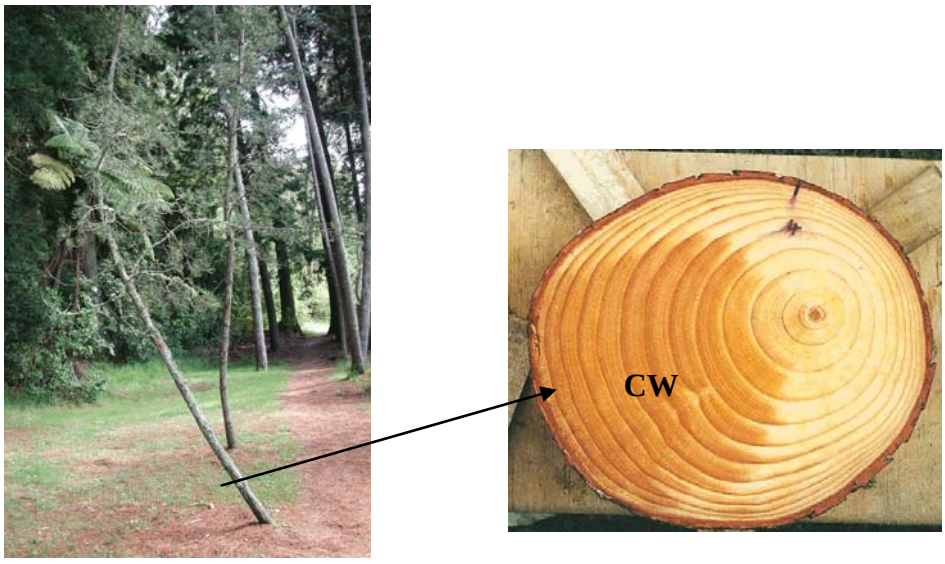


Figure 1.1 A leaning tree (a) and a cross-section of the leaning stem showing eccentric pith and wider growth rings in the compression wood (CW) region (b).

Compression wood is common in *Pinus radiata* (D. Don) (radiata pine or Monterey pine), which is the most important plantation-grown commercial species in New Zealand. *Pinus radiata* makes up about 89% of the total 1.82 million hectares of planted production forest in New Zealand (www.maf.govt.nz/statistics). It is easily affected by wind, and, like most fast-growing vigorous conifers, develops CW as a response to stem displacement or loss of a terminal leader (*3-5*). Inspection of log piles from almost any forest in New Zealand will reveal batches of logs in which CW occupies as much as 20% of the total log volume (*6*). Compression wood can thus make up a significant part of the merchantable product.

1.2 Effect of compression wood on wood utilisation

While CW is vital to the living tree, it is undesirable from the viewpoint of timber utilisation. Compression wood has a number of drawbacks which apply to *P. radiata* and other softwood species (*7,8*). A greater degree of longitudinal shrinkage (*3*) (3% or greater), compared to that found in normal wood (0.1- 0.3%) causes warp in sawn timber (*9,10*). Distortions caused by the differential shrinkage can be a particular problem when normal and CW both occur in the same board (Figure 1.2). The higher level of hardness and brittleness also makes timber with high levels of CW less useful for structural purposes. It is therefore desirable to detect and reject CW near the beginning of the production line. There are no commonly-used techniques for automatic detection of CW. Various imaging techniques are being tested for real-time detection of CW in sawn timber.

Figure 1.2 Warping of a stud in a fabricated wall due to compression wood (*7*)[1].

[1] Reproduced with kind permission of Springer Science and Business Media.

1.3 Compression wood in the pulp and paper industry

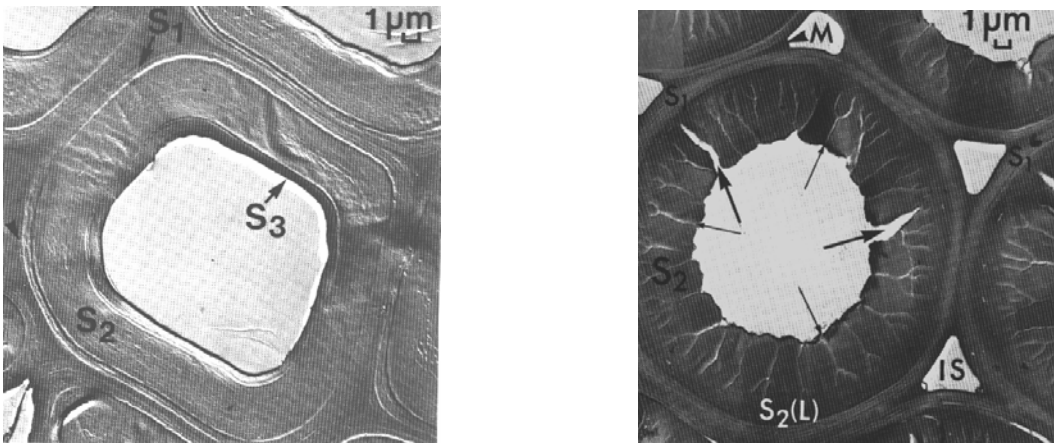
It has been claimed that CW in softwoods, including *P. radiata*, is not a desirable raw material for the pulp and paper industry. Kraft pulps prepared from CW gave lower yield, had higher Kappa numbers[2], poorer strength properties (due to fibre dimensions and fibre structure) and were more difficult to bleach than those from normal wood (*11-15*). However, the proportion of CW in wood supplied to any mill is generally considered to be too small to affect the quality of the pulp in a significant way (*12*). The presence of CW fibres in knotwood found in the whorl areas of the stem can result in excessive screen rejects during kraft pulping at high temperature or when thick chips from knotwood are employed. Rejects can be reduced by screening to remove oversize chips and/or by pulping at the lowest practical temperature (*16*).

1.4 Anatomy of compression wood

Compression wood differs from normal wood in its anatomical appearance (Figure 1.3). Key differences are (*17,18*):

- absence of a tertiary cell wall (S3) layer.
- presence of helical cavities and a higher microfibril angle in the secondary cell wall (S2) layer (up to 50% more than in normal wood).
- a much thicker and more highly lignified S2 layer.
- tracheids with a more rounded appearance in cross section.
- presence of intracellular spaces.
- tracheids shorter in length.

[2] Kappa number is a measure of lignin content in pulp.



(a) (b)

Figure 1.3 Cross-section of a normal wood tracheid (a), and a compression wood tracheid (b). Middle lamella (M); Primary wall (S_1); Secondary wall (S_2); Outer secondary wall (S_2(L)); Tertiary wall (S_3); Intercellular spaces (IS); Helical cavities (small arrows); Drying checks (large arrows) (*19*)[3].

All degrees of CW may be encountered from moderate forms to extreme types (*18,20*). Every sample may not have all of the features described above. In extreme cases, CW may develop all the features. Elsewhere there may be variability (e.g. Figure 1.4).

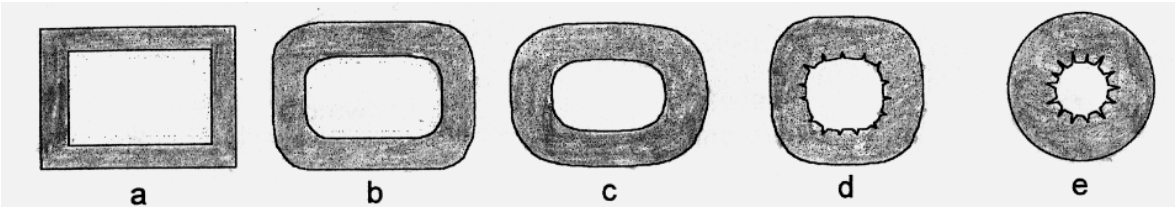


Figure 1.4 Cross-sections of softwood fibre cells, ranked for compression wood characteristics from (a) absent to (e) extreme. Intermediate forms are shown in (b), (c) and (d). Adapted from Newman (*21*).

Visual assessments are commonly used for detecting and/or classifying CW (as mild or severe) for research purposes. These can be subjective. Mild forms of CW may be indistinguishable visually from normal wood and must be identified under the microscope. Microscopic assessments are more accurate and reliable (*20*). Numerous other techniques including light transmittance through a thin disc, imaging, X-ray

[3] Reproduced with kind permission of Springer Science and Business Media.

scanning and spectral techniques have been used for detecting and classifying CW (see Sections 2.1.1 and 2.1.2) (*22-24*).

1.5 Chemistry of compression wood

There are significant differences in chemical composition between CW and normal wood. Compression wood contains:

- greater amounts of lignin and galactan
- smaller amounts of cellulose and galactoglucomannan (*19*) (Figure 1.5).

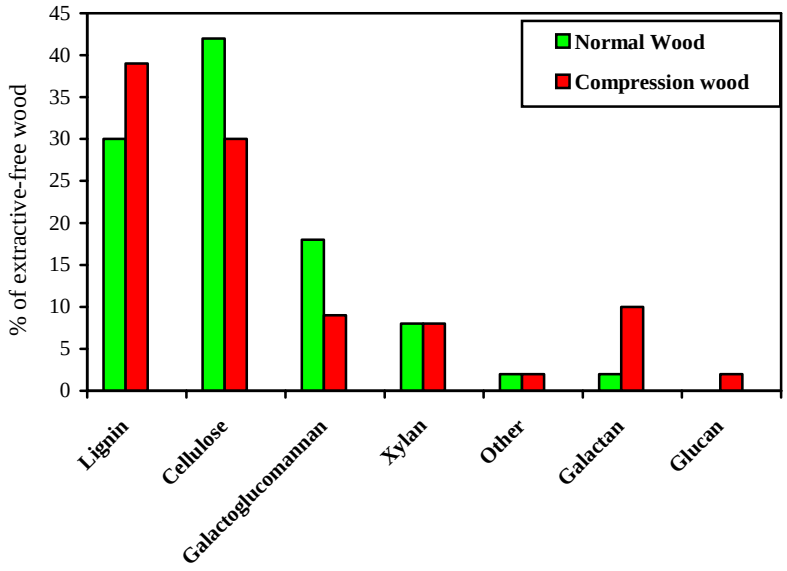


Figure 1.5 Typical chemical composition of normal wood and compression wood in conifers (*19*).

Not only does CW have a higher lignin content, but there are structural differences between CW and normal wood lignins. In particular, CW lignin contains a significantly higher *p*-hydroxyphenyl unit content, more carbon-carbon linkages between C9 units, a larger numbers of condensed units[4] and fewer β-O-4 inter-unit linkages (*19,25*). A more detailed description of the chemistry of CW is given in Section 2.5. While not well-studied, the chemistry of *P. radiata* CW is considered to be similar to that of other softwoods (*12,26-30*).

[4] For definitions on condensed and uncondensed units in lignin see Section 2.4.2.

1.6 Overall aim of this study

Differences in the chemistry of CW and normal wood suggest that they might be used for quantification of CW in a given wood sample. This could probably be done in two different ways. Firstly, a chemical test could be used to determine the amount of CW in an increment core or wood disc. Since these contain a mixture of normal wood and CW the same value for CW content could be obtained for a sample with a small amount of severe CW and one with a larger amount of milder forms of CW. The second method could be determination of the severity or the degree of CW development in a much smaller wood sample, such as one taken from a growth ring. Such a test would provide a valuable complement to microscopic methods, as it would have potential for determining an average CW content over a larger volume than is possible with microscopy.

The ultimate goal of this study was therefore development of a quantitative chemical test for the presence of CW and its severity. This might involve wet chemistry or spectroscopy. The intention was to develop the test for *P. radiata*, but it would be applicable to other softwood species, since the chemistry of CW is similar in all softwoods.

Few chemical methods for quantifying or classifying CW according to the severity have been reported. Thygesen and Meder (*31*) found that diffuse reflectance infrared fourier transform (DRIFT) spectroscopy can be used to differentiate *P. radiata* wood flour containing varying proportions of CW. Samples with 25% CW or more could be identified. Further work suggested that severity of CW could be determined from the galactose content, a correlation coefficient of 0.61 being reported (*32*). Newman *et al*. (*33*) recently reported a strong correlation between the galactose/glucose ratio and CW severity. While the results of these studies are encouraging, they do not define the potential for quantification of CW by chemical methods.

To assess the potential of a chemical test for CW, a comprehensive knowledge of the chemistry of CW is essential. In plantation-grown *P. radiata* all degrees of CW may be encountered in a continuum from near normal to severe forms. It is essential to know how the various chemical parameters change across this continuum and whether the rate of change is constant or variable.

To be useful, chemical characteristics that form the basis of the test must not be influenced by morphological origin of the wood sample (e.g. CW from stems, branches, knots, seedlings etc). In *P. radiata* the chemical composition of the cell wall varies with age and position within the tree (*34*,*35*). Knowledge of the effect of this variability on the chemistry of CW is essential. To the best of the author's knowledge, the impact of these factors on the chemistry of *P. radiata* CW has not been investigated.

The work described in this thesis aimed to:

1. Determine how the composition and lignin structure of CW in *P. radiata* changes with its severity, i.e., from normal through mild to severe CW.
2. Establish how the chemistry of CW varies in wood samples from different morphological structures of *P. radiata.*
3. Determine how the chemistry of CW in *P. radiata* changes with wood age.

Twenty seven CW and normal wood samples of *P. radiata* were collected. These included juvenile wood, mature wood, earlywood, latewood, branches, knots, 1-year and 2-year old seedlings. As it is known that the chemical composition of normal wood in *P. radiata* varies with wood age and the location within the tree (*35*), each CW sample was matched with a sample of wood from the same growth ring on the opposite side of the stem. This allowed the separation of the effects of CW development from those associated with wood age.

The degree of CW development was determined by fluorescence microscopy (*36*). It was only possible to describe three categories by this method: normal; mild; and severe. Finer distinctions were not possible because the anatomical features of mild CW are highly variable and samples of sufficient size and uniform appearance could not be obtained by microscopy.

Analysis for the following components was carried out on all samples (Figure 1.6):

- Klason and acid-soluble lignins using standard methods.
- Neutral monosaccharides (arabinose, galactose, glucose, xylose and mannose).
- Uncondensed β-ether units and the relative proportion of *p*-hydroxyphenyl and guaiacyl C9 unit products by thioacidolysis (*37*).

- Degree of condensation of lignin by thioacidolysis combined with either Size Exclusion Chromatography (SEC) (*29*,*38*) or ^{31}P NMR spectroscopy (*30*,*39*).

Pyrolysis-GC-MS, was also used as a rapid method of lignin characterisation (*40-42*).

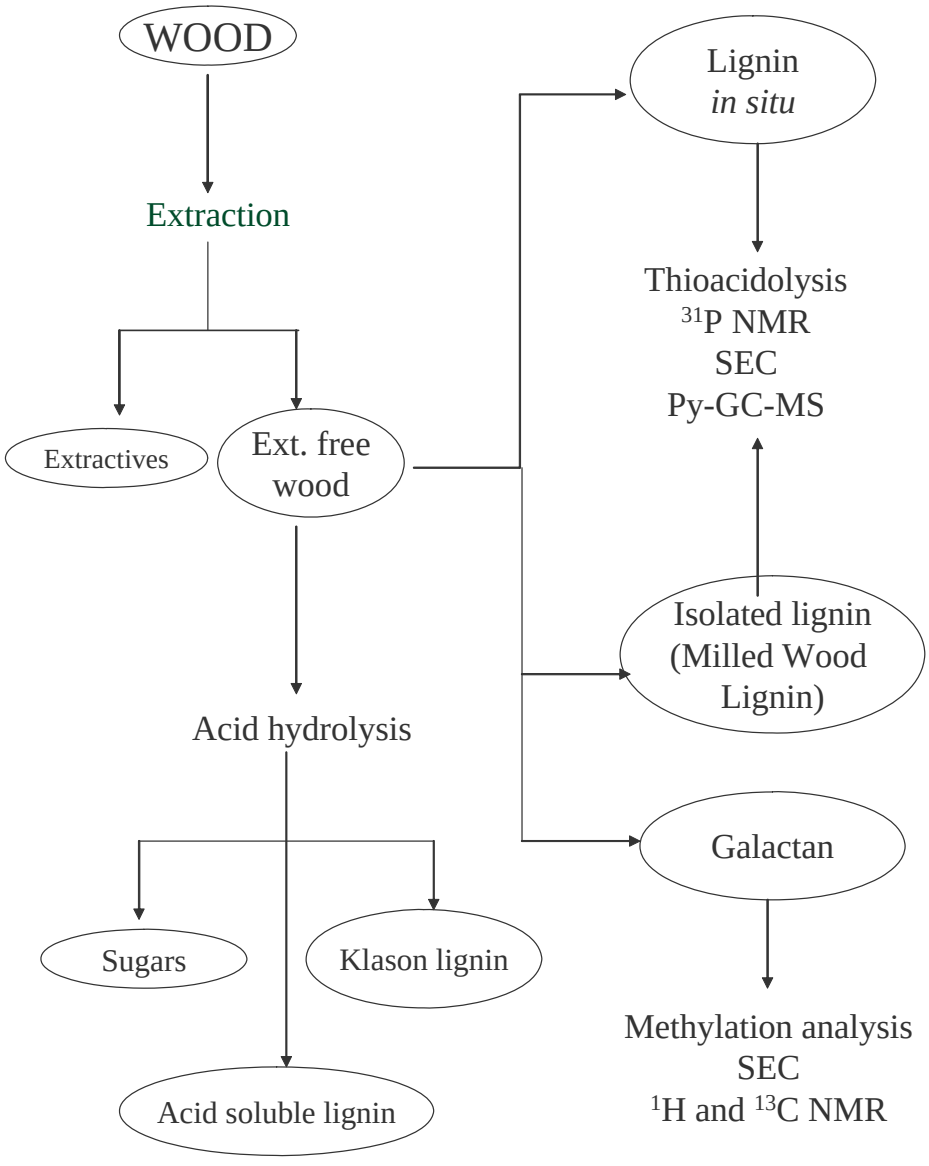


Figure 1.6 Flow diagram showing the analyses scheme.

1.7 Thesis organisation

A review of literature is presented in Chapter 2. Chapters 3 and 4 describe the bulk of the experimental work carried out in the thesis. Chapter 3 focuses on the variability of chemical components within a single disc (samples cut transversely across the stem). The relationship between chemical composition and severity of *P. radiata* CW is explored in Chapter 4.

The remaining chapters cover a number of additional investigations carried out as a part of this study:

- Chapter 5 contains a discussion about the isolation and characterisation of CW galactan from *P. radiata*.
- In Chapter 6 the lignin structure of isolated cell wall layers and *P. radiata* callus tissues is described. This Chapter relates to non-CW samples.
- Chapter 7 presents a new method for determining the degree of condensation of phenolic and etherified C9 units in *in situ* wood lignins.

Chapters 3, 4 and 7 are written as draft manuscripts for publication in scientific journals. This necessitates reiteration of a certain amount of introductory material.

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CHAPTER 2

2 Literature Review

This chapter reviews literature relating to the characteristics of compression wood with the main focus on its chemistry.

2.1 Introduction

Several reviews on work focusing on CW have been produced (*1,2*). The most comprehensive is the three-volume treatise by Timell (*3*), who was one of the pioneering investigators in the field. The facts below are derived from Timell's review unless otherwise stated. An introduction to the subject of CW, including chemical, physical and anatomical properties can be found in many other publications (*4-10*).

Compression wood formation in softwood conifers has been recognized since the 18th century. According to Timell, Carl von Linné, during his travel to the north of Sweden, noticed that crooked pines had hard and dark wood, on their lower side. He described this as "box wood". German botanist Karl Gustav Sanio (1860) was the first to describe the anatomical structure of CW (*1,11*).

Compression wood appears to be formed in all three gymnosperm genera, Coniferales, Taxales and Ginkoales (*12*). Tissues resembling CW have also been observed in a few primitive woody angiosperms (hardwoods) in which the xylem consists largely of tracheids e.g. *Buxus sempervirens* (*13*).

Compression wood is formed on the underside of leaning trees and branches. Its effect is manifested by a slow change in the orientation of the stem or branch. Formation of CW is generally considered to be a gravitational response brought about by the redistribution of auxins (*6*). Compression wood is more readily produced in fast-growing than in slow-growing trees, and is probably more

widespread than generally thought. There is hardly a forest or plantation tree that does not have some CW in its stem (*14*).

Compression wood occurs in several month old seedlings (*15*), which are highly susceptible to wind. Here it occurs in patches throughout the stem rather than on one side. Compression wood is more common in juvenile wood than in mature wood.

Mild CW formation is common in fast-growing conifer species such as *Pinus radiata*. It can be found:

- on the lower side of the stems of slightly-leaning trees
- on the lower side of tree stems that have been strongly tilted for a short period
- on one side of strongly-leaning trees
- in the transition zone between normal wood and CW (*16*).

The chemical composition and anatomical features of CW influence its physical properties (*17*). Compared to normal wood CW has:

- increased longitudinal shrinkage (Section 1.4)
- higher microfibril angles (Section 2.7.1.1)
- greater density
- reduced permeability
- a slightly lower modulus of elasticity.

2.1.1 Methods for detecting compression wood

2.1.1.1 Visual assessment

In green wood (undried timber) CW appears dark reddish in colour, and this gives rise to names such as “redwood”, “rotholz” and “bois rouge”. Compression wood appears darker both because it absorbs more light (due to the higher lignin content) and scatters less light (due to the thicker tracheid walls). Mild and moderate forms are paler in colours and more difficult to detect. Wetting enhances the colour of all CW. An eccentric pith and wider growth rings are indirect evidence of CW (Figure

1.1b). Visual detection in combination with a planimeter[1] can be used to determine the CW content in the cross section of a log.

2.1.1.2 Microscopy

Microscopy is the most accurate method for detecting CW. It allows a clear distinction to be made between mild and severe forms (*18,19*).

Among the anatomical features usually associated with severe CW are rounded cells with a highly lignified outer S2 layer occurring right round the periphery of the cell wall, intracellular spaces, the absence of an S3 layer and the presence of helical checks (Figure 2.1).

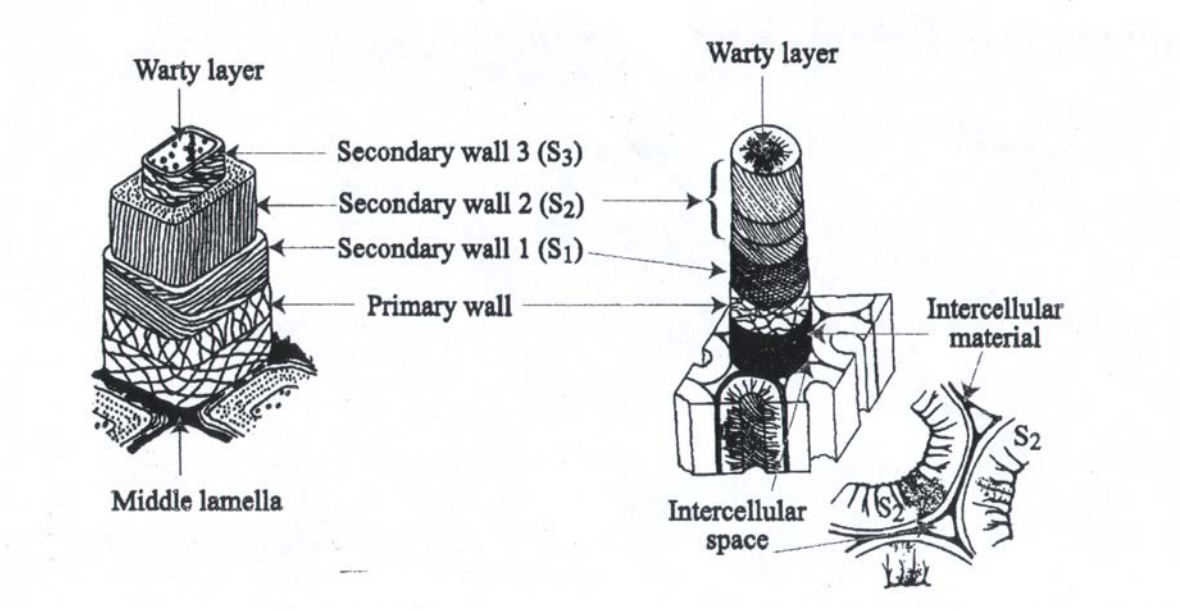


Figure 2.1 Schematic model of the cell-wall structure of (i) a softwood tracheid ; (ii) a typical compression wood tracheid (*7,20*)[2].

A study of variation of anatomical features of CW by Yoshizawa *et al*. (*21*) in 38 gymnosperm species showed that all the above features, except helical checks, were present in CW of all gymnosperms species studied. These authors suggested that helical checks are not an essential feature of CW formation.

Mild CW formation exhibits more variable anatomical features than severe CW. In mild cases tracheids resemble those of normal wood in their four-sided cross-

[1] The planimeter is a mechanical instrument used to compute the area of a planar region.

[2] Reproduced with kind permission of Walter du Gruyter and Springer Science and Business Media.

sectional shape with slightly rounded appearance (*21*). According to Yumoto *et al*. (*16*), the first feature to appear in CW formation is increased lignification of the outer S2 region of the cell wall. This occurs first in the corners and later a continuous layer is formed. Mild cases of CW formation in *P. radiata* have been studied by Donaldson *et al*. (*18*), who found that the absence of a S3 layer and the presence of intercellular spaces were variable features of the mildest forms. Further, there was a lot of variation in the degree of lignification in the cell walls. Singh *et al*. (*22*) have reported the presence of a distinct S3 layer in the tracheids in cases of mild CW formation in *P. radiata.*

2.1.1.3 Other methods

The presence and proportion of CW in thin (2-3 mm) wet cross-sections of a stem can be determined by use of transmitted light (*23*). This method is based on the fact that CW is opaque to transmitted light, whereas normal earlywood and latewood are almost translucent. The method can detect mild forms of CW and is particularly useful for ascertaining the presence of small amounts of CW within larger areas of normal wood (*14*).

Anderson and Walter (*24*) employed digital image analysis to differentiate grades of CW severity in colour images of wood discs. Mild CW gave a light orange to reddish colour, whereas severe CW was dark brown in transmitted light (Figure 2.2).

Moëll and Fujita (*25*) have tried using roundness of lumen cell shape for detection of CW. While severe CW was successfully identified, this method did not detect mild CW adequately.

Hagman and Nyström (*26,27*) used spectral imaging and multivariate modelling for non-destructive assessment of CW. They reported that this technology can be used for real-time mapping of CW on dry wood surfaces.

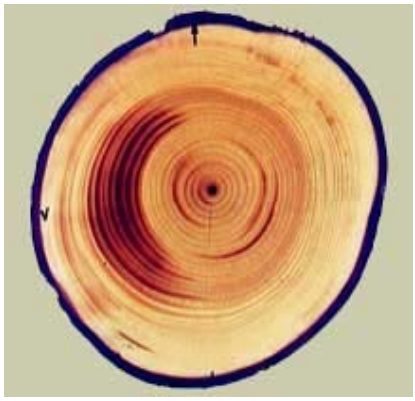


Figure 2.2 Darker compression wood revealed by use of transmitted light on a thin cross-section of a stem. Adapted from website http://www.forestry.uk. Retrieved on 20.06.2003.

Nyström (*28*) examined the effectiveness of four different approaches - spectral imaging, colour scanning, X-ray scanning and tracheid effect scanning - in the detection of CW and spiral grain and the predicting of distortion. A combination of tracheid effect scanning and colour scanning was found to be best for detecting CW in both green and dry material (*29*). Nyström and Kline (*30*) used X-rays in combination with colour photography to detect surface CW. They developed a multivariate regression model based on colour information which explained 87% of the variation in a small number of southern pine samples.

Techniques such as infrared (IR) and near infrared (NIR) spectroscopy have also been used to detect CW with mixed success (*31*,*32*).

2.1.2 Classification of severity

A number of attempts have been made to classify the severity of CW formation (Table 2.1).

Table 2.1 Methods used to classify severity of compression wood development.

Method	No. of grades determined	Description of grades	Reference
Light transmittance	2	Mild Pronounced	(*23*,*33*)
Light transmittance	3	Slight Moderate Strong	(*34*)
Visual	3	Slight Intermediate Pronounced	(*35*)
Light transmittance/ planimeter	3	Borderline Intermediate Severe	(*36*)
Light transmittance	3	Slight Moderate Pronounced	(*37*)
Visual	3	Slight Moderate Severe	(*38*)
Microscopy	5	0 = Normal wood 1-3 = Mild CW 4 = Severe CW	(*39*)
Light transmittance	6	a. Normal wood b. Latewood partly opaque c. Latewood generally opaque d. Latewood and earlywood partly opaque e. Latewood opaque and earlywood generally opaque f. Latewood and earlywood highly opaque	(*40*)
Visual	4	a. Normal b. Small crescents of patches of dark wood - not more than half of the width of the growth ring c. 45º of an arc - not entire radial width of increment consists of dark wood d. Dark ring covers entire radial width	(*41*)
Microscopy	6	Based on anatomical features from microscopic studies	(*16*)

2.1.3 "Opposite" wood

The wood produced on the upper side of a branch which has formed CW or on the side of a stem opposite to the site of CW formation is referred to as "opposite" wood (OW) (Figure 2.3). Many early investigators, in discussing the properties of CW, made comparisons with wood formed in the opposite side of the same growth ring. According to Timell (*42-44*), the chemical composition of OW is indistinguishable from that of normal wood, but there is a slight difference in structure, latewood tracheids in OW having a thick and highly-lignified S3 layer.

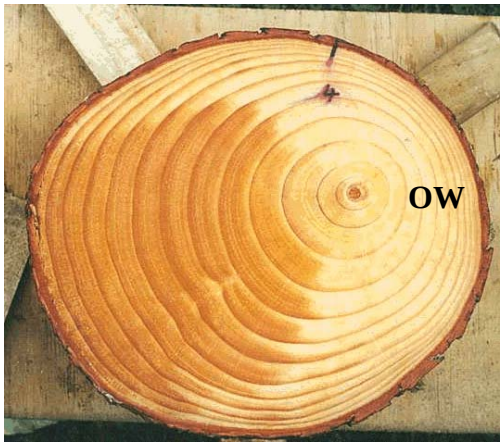


Figure 2.3 Location of opposite wood (OW) in a stem containing compression wood.

2.2 Chemistry of compression wood

Chemical components of CW and normal wood, as reported by Côté and coworkers (*45*) and Timell (*46*), are presented in Table 2.2. Compression wood differs from normal wood in having a higher lignin content, fewer glucose residues, and higher levels of galactose residues. It contains fewer acetyl, mannose, arabinose and xylose residues than normal wood.

The chemical characteristics of *P. radiata* CW are very similar to those of CW in other softwoods (*47-50*). Lignin contents of up to 34.4% were reported for *P. radiata* in these investigations. More recently, other authors have reported lignin content ranging from 31 to 35% (*51-53*). The variation in reported lignin content is

presumably related to the severity of CW in the samples studied. The lignin content increases in the order mild to moderate to severe CW (*54*).

Table 2.2 Average composition of normal wood (NW) and compression wood in 27 gymnosperm species (*54*) and *P. radiata* (*51*). (Values are expressed as percentage of the dry weight of extractive-free wood).

Component	Average for softwoods		*P. radiata* [1]		
	NW	CW	Corewood	Slabwood	CW
Lignin	30.1	38.9	26. 1	26.5	31.3
Acetyl	1.31	0.84	-	-	-
Uronic anhydride	4.7	4.5	2.2	1.3	1.1
Galactose	2.1	10.3	2.6	2.8	8.6
Glucose	43.0	32.5	43.6	48.7	42.4
Mannose	9.8	5.3	13.6	13.5	9.8
Arabinose	1.6	0.9	2.5	1.2	1.3
Xylose	7.1	6.6	9.3	6.0	5.5

[1] Monomer sugar contents were recalculated as a percentage of wood from (*51*) where it was expressed as a percentage of total sugars.

2.3 Lignin

2.3.1 Introduction

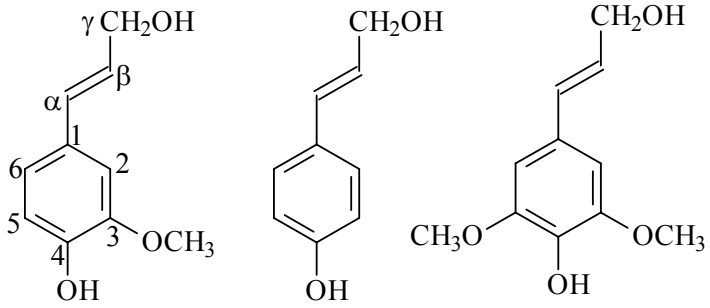
Lignin represents one third of the land biomass and is found in all terrestrial vascular plants (*55*). Incorporation of lignin into the cell walls of plants increases mechanical strength and, limits penetration of water into wood cells. Woody tissue is therefore more compact and resistant to attack by microorganisms. The lignin in wood helps to bond the cellulose microfibrillar skeleton into a coherent whole (*4*).

The high lignin content of CW influences its physical and mechanical properties. The characteristic red colour has been attributed to the higher lignin content (*54*). The higher lignin content and the more condensed lignin result in greater rigidity. This is probably an adaptation to growth stress (*46,56*). Okuyama *et al*. (*57*) have reported a positive correlation between lignin concentration in the secondary wall and growth stress resulting in CW formation.

Remarkable differences exist between CW and normal wood lignins (*54*). To assist the understanding of the structural differences between CW and normal wood lignins, a brief overview of softwood lignin structure is given below. More details can be found in references (*55,58-61*).

2.3.2 Lignin structure

Lignin is a complex three-dimensional polymer consisting of phenylpropane (C9) units linked together by a variety of carbon-carbon and carbon-oxygen bonds. There are three hydroxycinnamyl alcohols or monolignols which undergo oxidative coupling reactions to form lignin (Figure 2.4). Coniferyl alcohol (3-methoxy-4-hydroxycinnamyl alcohol), **1**, is the predominant lignin precursor in almost all softwoods, together with a small amount (<5%) of *p*-coumaryl alcohol (4-hydroxycinnamyl alcohol), **2**. It can be classified as a guaiacyl or a G-lignin. Compression wood lignins contain up to 30% *p*-hydroxyphenyl (H) units derived from *p*-coumaryl alcohol (*62*), so they can be classified as G-H lignins. Both coniferyl alcohol and sinapyl alcohol (3,5-dimethoxy-4-hydroxycinnamyl alcohol), **3**, are building blocks of hardwood lignins.



1 coniferyl alcohol

2 *p*-coumaryl alcohol

3 sinapyl alcohol

Figure 2.4 Lignin precursors (the conventional lignin labelling scheme is shown in **1**).

2.3.3 Biosynthesis of lignin

2.3.3.1 Monolignol formation

Lignins and other phenylpropanoids such as flavonoids are synthesised in plants via a phenylproponoid pathway (*61,63*). This consists of several steps which are shown in Figure 2.5.

The sequence can be summarised thus:

I. D-glucose is converted to shikimic acid.
II. Shikimic acid is converted to two aromatic amino acids: L-phenylalanine and L-tyrosine.
III. Deaminating enzymes convert these amino acids to cinnamic acids.
IV. Stepwise hydroxylation by hydroxylases and eventual methylation by *O*-methyltranferases transform the cinnamic acids to hydroxycinnamic acids (*p*-coumaric, ferulic and sinapic acids).
V. Hydroxycinnamic acids are reduced to the corresponding cinnamyl alcohols by the successive action of three enzymes.

It has been reported that the activities of 4CL, PAL, COMT and CAD (Figure 2.5 and Table 2.3) are 1 to 3-fold higher in CW xylem than in normal wood xylem (*64-66*). Therefore, it is likely that compressional stress induces, in various degrees, the expression of most genes involved in the biosynthesis of monolignols. This results in overall augmentation of the synthesis of lignin in CW tissues (*65*).

This does not explain the preferential increase in *p*-coumaryl alcohol which generates *p*-coumaryl-enriched lignin in CW. Zhang and Chiang (*65*) have reported that 4CL activity toward 4-coumaric acid was increased in the xylem of loblolly pine CW. They speculated that the 4CL enzyme, with altered activities toward different substrates, could regulate the distribution of cinnamic acid derivatives into various phenylproponoid pathways, favouring a characteristic flux of precursors for the formation of *p*-hydroxyphenyl-enriched lignin in CW tissue.

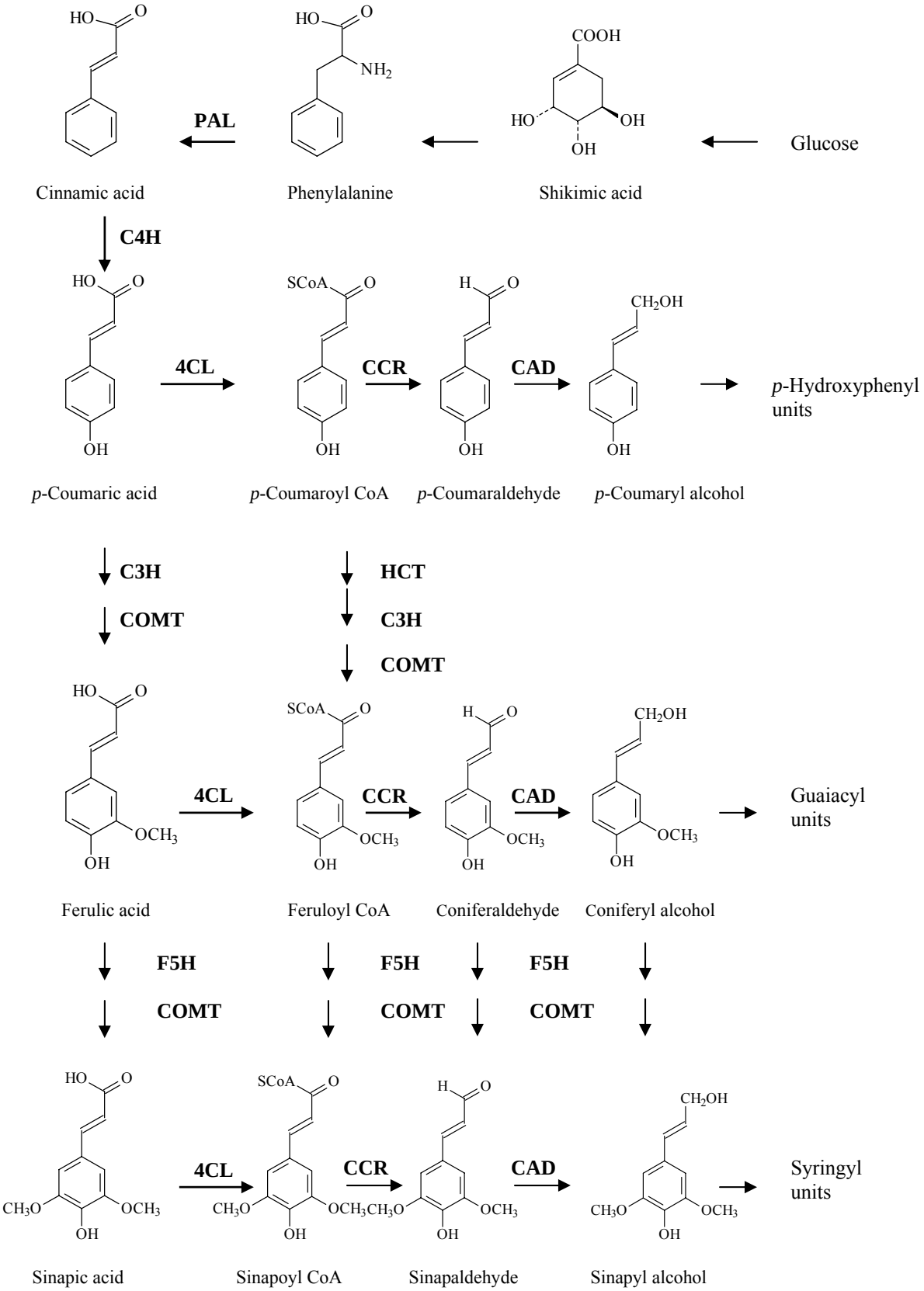


Figure 2.5 Simplified metabolic pathway to the formation of lignin precursors (*63*). The enzymes involved are listed in Table 2.3.

Table 2.3 Enzymes involved in the lignin precursor metabolic pathway.

Enzyme	Abbreviation
Phenylalanine ammonia-lyase	PAL
Cinnamate 4-hydroxylate	C4H
p-Coumarate 3-hydroxylase	C3H
Hydroxycinnamoyl CoA	HCT
Caffeic acid/5-hydroxyferulic acid *O*-methyltransferase	COMT
Ferulate 5-hydroxylase	F5H
4-(Hydroxy)cinnamoyl CoA ligase	4CL
Cinnamoyl CoA reductase	CCR
Cinnamyl alcohol dehydrogenase	CAD

The effect of *p*-hydroxyphenyl-enriched lignin on the role of CW in the living tree is not documented. Nevertheless, it is speculated (*54,67*) that *p*-hydroxyphenyl moieties have greater potential for coupling at both C3 and C5 positions in the ring during radical polymerisation. This would result in formation of a more condensed lignin network, which might help to withstand compressive stress.

Various mutant and transgenic plants in which the steps in the monolignol biosynthetic pathway are regulated show deviations from the normal lignification process. For example, down-regulation of genes encoding the C3H enzyme in alfalfa has been shown to increase *p*-coumaryl levels by up to 65% (*68*).

2.3.3.2 Polymerisation of p-hydroxycinnamyl alcohols

Freudenberg *et al*. (*55*) found that when coniferyl alcohol was treated with a fungal laccase or horseradish peroxidase, a higher molecular weight polymer known as dehydrogenation polymer (DHP) was formed. Laccase, an oxygen-dependent oxidase, and peroxidase, an H_2O_2-dependent haemoprotein, both oxidise monolignols to phenoxy radicals (*55,61*). Freudenberg and his co-workers suggested that lignin is formed through coupling of phenoxy radicals (dimer formation). Dehydrogenation polymers are synthetic lignins, widely used as lignin model compounds (*69*).

The polymerisation of lignin is initiated by enzymatic dehydrogenation of monolignol. This gives rise to phenoxy radicals having five main mesomeric forms

(Figure 2.6). Only four of these (a-d, in Figure 2.6) are involved in lignin biosynthesis. Form (e) is sterically and thermodynamically disfavoured (*60*). The phenoxy radicals then couple non-enzymatically to produce quinonemethides as reactive intermediates (*55*) (Table 2.4). The relative electron density of the different phenoxy radicals determines, in part, the frequency of involvement of the different sites in coupling reactions.

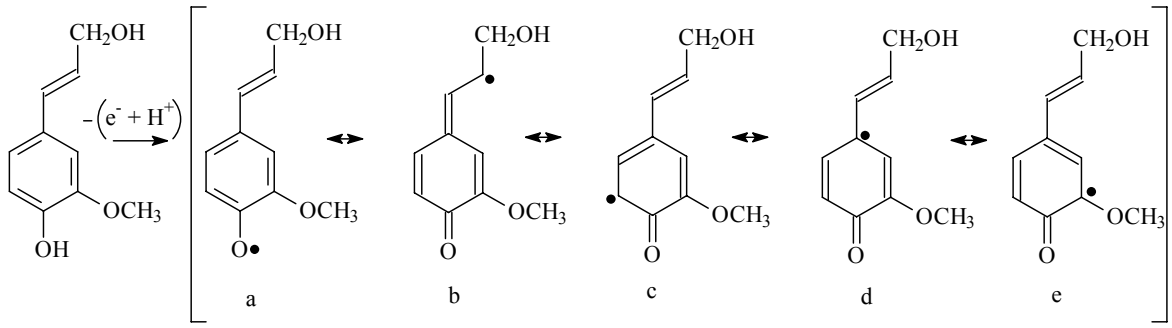


Figure 2.6 Mesomeric forms of phenoxy radicals derived from coniferyl alcohol during lignin biosynthesis.

Table 2.4 Coupling modes in dimer formation from coniferyl alcohol (*60*).

	a	b	c	d
a	Unstable peroxide	β-O-4	4-O-5	1-O-4
b	β-O-4	β-β	β-5	β-1
c	4-O-5	β-5	5-5	1-5
d	1-O-5	β-1	1-5	1-1

Figure 2.7 shows the β-O-4 radical coupling of two coniferyl alcohol radicals (Figure 2.6a and b) resulting in the β-O-4 quinonemethide intermediate. Nucleophilic addition of water to the quinonemethide yields an arylglycerol-β-aryl ether structure.

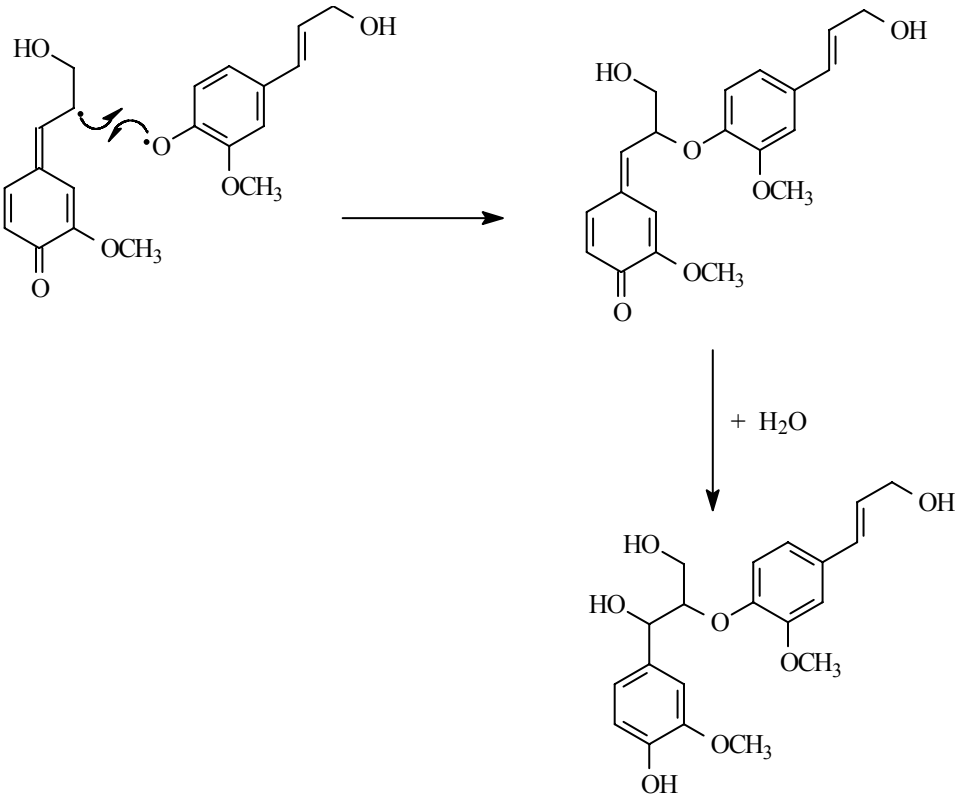


Figure 2.7 Proposed mechanism for the formation of β-O-4 linkages (*59*).

After formation of the oxidatively-coupled dimers, further aryloxy radicals are generated. These new radicals couple oxidatively by end-wise or by bulk polymerisation. Sarkanen (*70*) suggested that the distribution of inter-unit linkages in lignin depends on the type of polymerisation.

End-wise polymerisation

In end-wise polymerisation, monolignols are added to the initial dimer one by one and cross-products are formed (Figure 2.8) (*70*). This process is accelerated at low concentrations of coniferyl alcohol, and leads to higher proportions of β-O-4 and β-1 type coupling. The limited number of coniferyl alcohol and coniferaldehyde end-groups found to be present in Björkmann lignin isolated from spruce indicates that this type of polymerisation predominates in softwoods (*60*).

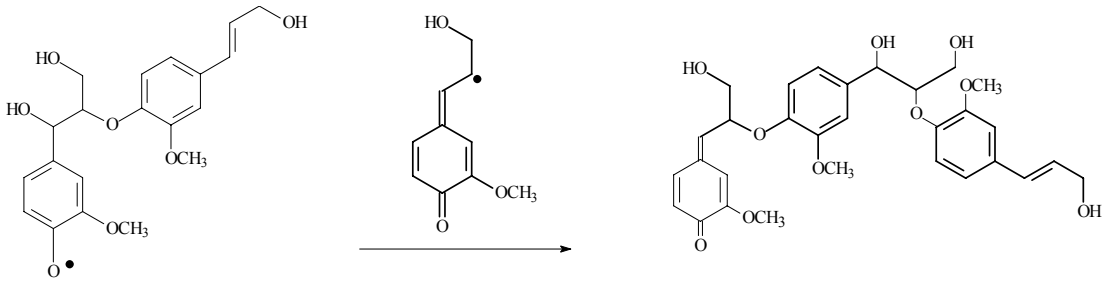


Figure 2.8 An example of end-wise polymerisation: formation of a β-O-4 linkage.

Bulk polymerisation

In bulk polymerisation the lignin polymer is formed by the coupling of end group radicals of two oligo-lignols (Figure 2.9). This process is accelerated at high concentrations of coniferyl alcohol and leads to higher proportions of β-5, 5-5 and 4-O-5 type couplings in the resulting polymer (*59,70,71*).

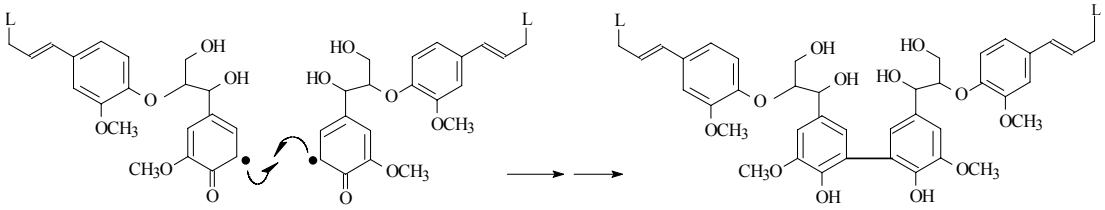


Figure 2.9 An example of bulk polymerisation: formation of a 5-5 linkage. L = Lignin.

A detailed analysis of *Pseudotsuga menziesii* CW lignin (*62*) showed a greater proportion of 5-5 and β-5 type linkages than in normal softwood lignin, but fewer β-O-4 and β-1 linkages. On the basis of this observation, it has been suggested that CW lignin formation occurs through bulk polymerisation due to the presence of high monolignol concentrations (coniferyl alcohol and *p*-coumaryl alcohol radicals) in the cell wall (*54*). This theory has been questioned, and is discussed further in Section 2.5.4.2.

Inter-unit linkages found in softwood lignins are shown in Figure 2.10. A recently developed diagrammatic representation of the structure of softwood lignin is given in Figure 2.11.

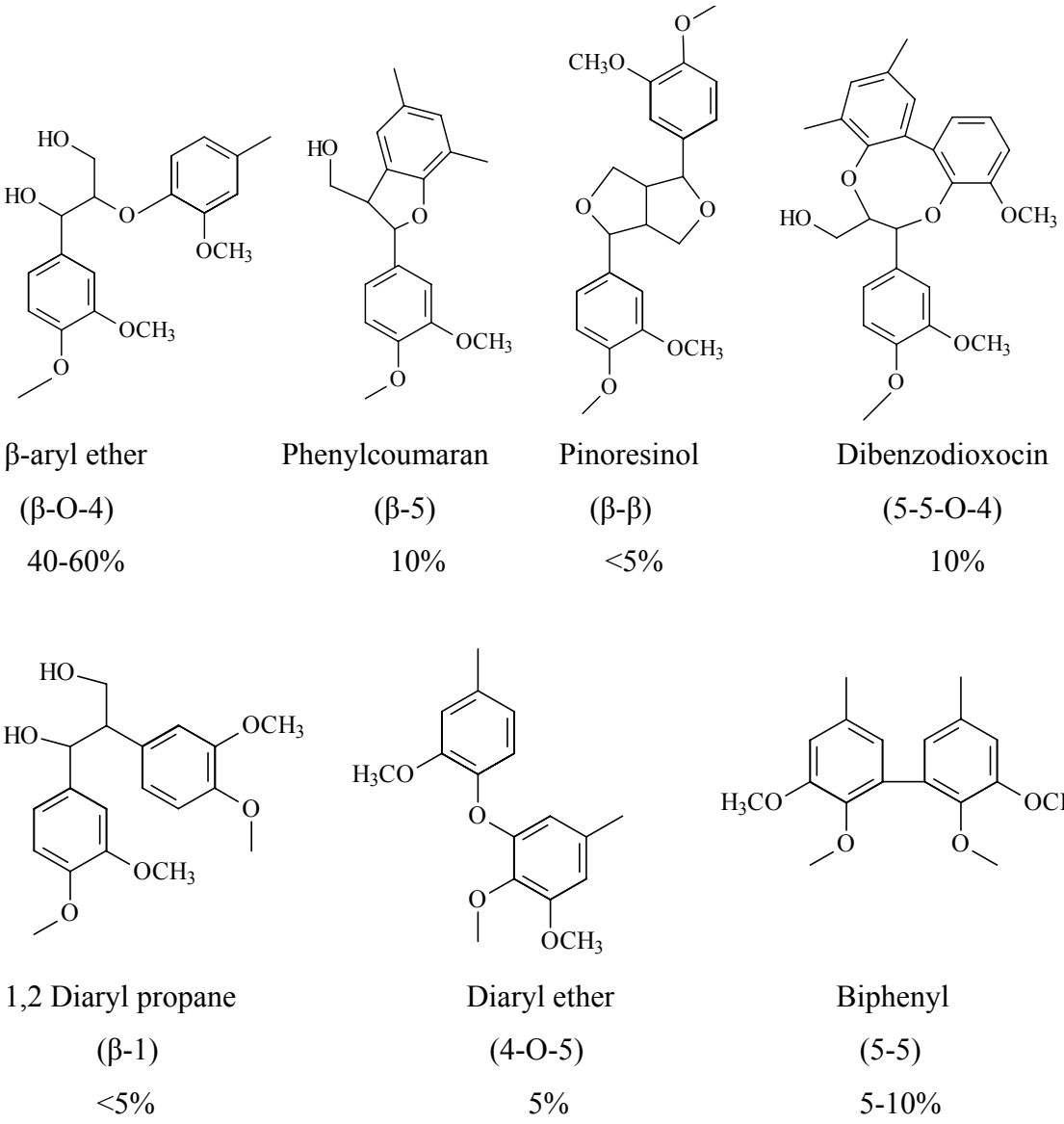


Figure 2.10 Inter-unit linkages in softwood lignins (*59,71,72*).

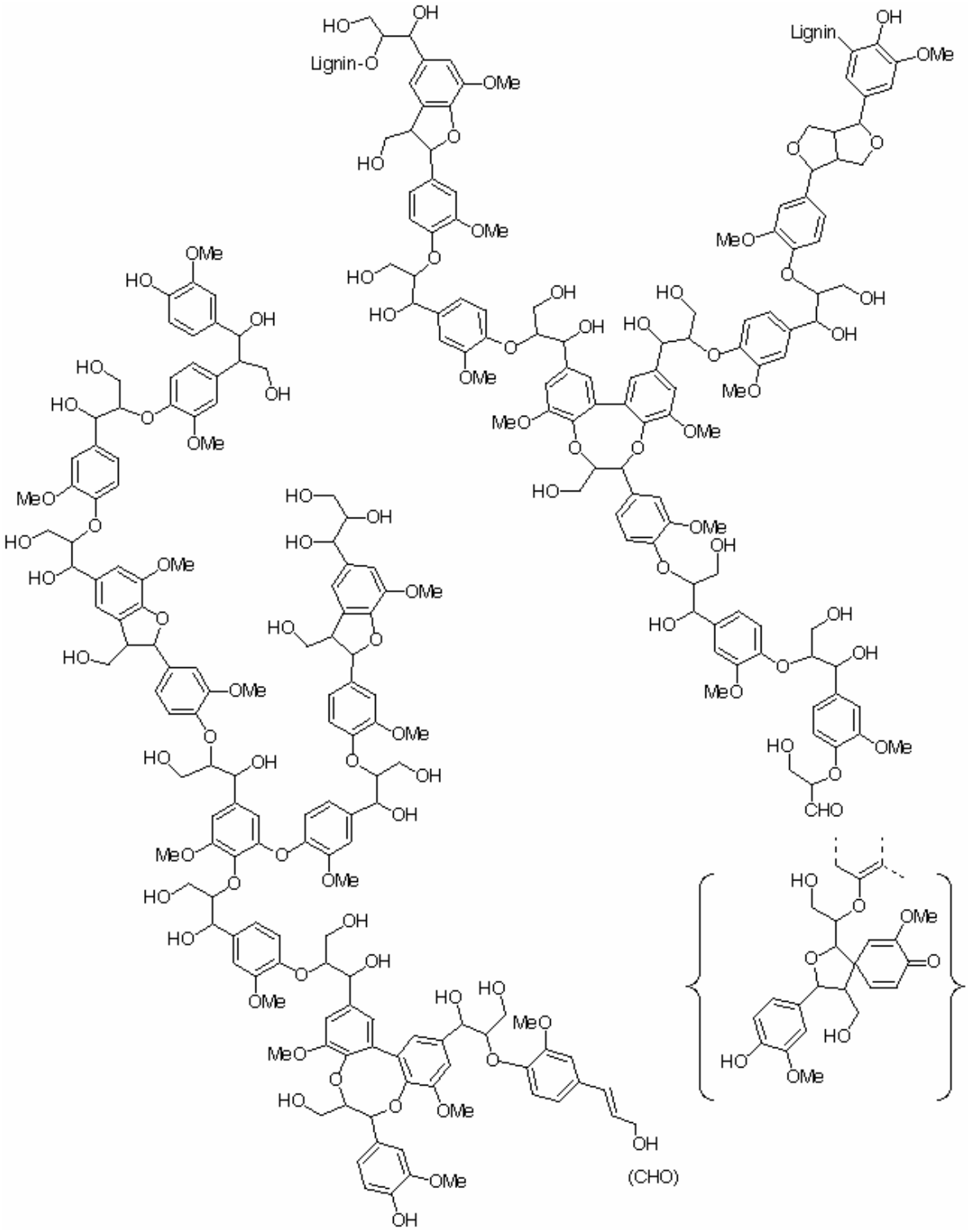


Figure 2.11 A structural scheme for a fragment of softwood lignin. Adapted with permission from website http://www.helsinki.fi/~orgkm_ww/lignin_structure.html. Retrieved on 28.04.2006.

2.4 Characterisation of lignin

2.4.1 Isolated lignin or milled wood lignin

Many lignin characterisation methods, especially those based on NMR spectroscopy, require isolated lignin samples in order to overcome interference from carbohydrate components in the cell wall.

The most commonly used procedure for isolating lignin from wood involves vibratory ball-milling of wood meal under nitrogen in order to destroy the cell wall structure, followed by extraction of a portion of the lignin with a dioxane-water mixture (*73*). The extract is known as milled wood lignin (MWL). It is generally considered that MWL is derived from the secondary wall lignin (*74,75*). Although MWL is a very useful preparation that has been used as the basis of hundreds of studies on lignin structure, the yields are at most only 20% of the Klason lignin.

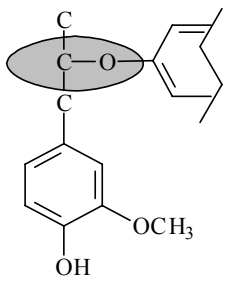
It has not been possible to isolate lignin from plant tissues without causing structural changes. Ball-milling increases the phenolic hydroxyl and α-carbonyl content of the lignin, probably due to homolytic cleavage of β-O-4 linkages (*76*).

2.4.2 Condensed vs. uncondensed units in lignin

As discussed in Section 2.3.3.2, lignin consists of C9 units held together by different types of linkages. Some of these are easier to break than others. For example, during wood pulping α- and β-ethers are cleaved, whereas 5-5, 4-O-5, β-β, and β-1 linkages remain relatively inert. For practical reasons, it is useful to know how easily a lignin will breakdown under a given set of conditions, i.e. how "condensed" the lignin may be. This concept has been extended in order to identify **uncondensed** C9 units which are easily cleaved and **condensed** C9 units which are unreactive (*76*).

While these concepts have obvious practical utility, it is difficult to specify what is meant by the terms "condensed" and "uncondensed" C9 units since each non-terminal C9 units has at least 2 inter-unit linkages. The following definitions have been suggested:

Uncondensed units: Structures in lignin in which the side chain is connected with the next unit through an easily-cleaved linkage such as β-O-4, **4** or α-O-4, **5**. These links are amenable to hydrolysis reactions catalyzed by acids and bases.



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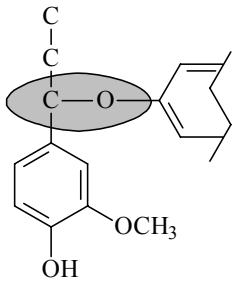

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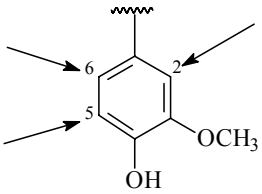
Condensed units: Structures in lignin in which C2, C5 and C6 in the ring of one C9 unit form covalent links with carbon or oxygen in a second C9 unit, **6**. Examples of such linkages involving C5 are β-5, 4-O-5, 5-5-O-4, 5-5. Structures with condensed linkages at C2 and C6 are less frequent.



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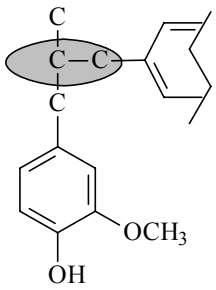

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β condensed units: Structures in lignin in which the β position in the ring of one C9 unit develops a covalent link with carbon in a second C9 unit, **7**. Examples of such linkages are β-β and β-1.

In practice, the definitions tend to vary with the method used for the determination of the relative proportions of the structures.

2.4.3 Methods used for determining the uncondensed and condensed units in lignin

Characterisation of lignin uses isolated lignin samples (MWL) and uses either NMR spectroscopy or degradative methods in which the macromolecule is chemically or thermally degraded to its constituent building blocks. The resulting products are analysed by gas chromatography (GC) and/or mass spectrometry (MS). Information

about inter-unit linkages and side chain structures of the lignin macromolecule is gained from the degradation products.

The most commonly-used methods to identify the proportion of C9 units degraded to monomeric C9 units are ethanolysis, thioacidolysis or DFRC (derivatisation followed by reductive cleavage). The lower the proportion of monomeric degradation products the more condensed the lignin. Table 2.5 summarises other methods used to measure the proportion of condensed and uncondensed C9 units in lignin.

Table 2.5 Methods used to determine the proportions of uncondensed and condensed units in lignin.

Method	Inter-unit linkage measured	Reference
Nitrobenzene oxidation	β-O-4, α-O-4	(*77*)
Ethanolysis	β-O-4	(*78*)
Thioacidolysis/GC	Releasable β-O-4	(*79,80*)
DFRC (Derivatisation followed by reductive cleavage)/GC	Releasable β-O-4	(*81*)
Ozonolysis	β-O-4	(*82,83*)
Oxidative degradation	Condensed at C5, 6 (phenolic only)	(*84*)
Thioacidolysis/desulfurisation	Condensed at C2, 6, 5 and β cond.	(*85*)
Thioacidolysis/size exclusion chromatography	Condensed at C2, 6, 5 and β cond.	(*86*)
Thioacidolysis/^{31}P NMR	Condensed at C5 only	(*87*)
^{1}H NMR	Condensed at C2, 6, 5	(*88-90*)
^{13}C NMR	Condensed at C2, 6, 5	(*91-93*)

2.4.3.1 Nitrobenzene oxidation

This method involves oxidation of isolated or *in situ* lignin with nitrobenzene in hot aqueous alkali. The reaction cleaves the side chains of β-O-4 and α-O-4 structures in lignin (*59,77*) (Figure 2.12).

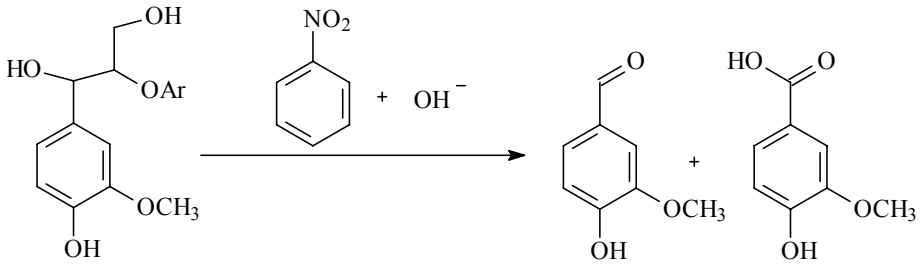
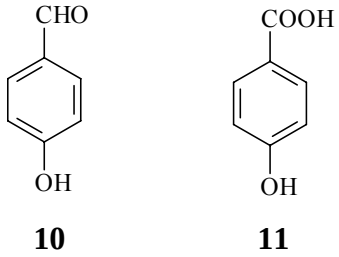


Figure 2.12 Nitrobenzene oxidation of lignin.

While the main product derived from guaiacyl lignin is vanillin, **8**, small amounts of vanillic acid, **9** are also produced. Likewise *p*-hydroxyphenyl lignins produce *p*-hydroxybenzaldehyde, **10**, and small amounts of *p*-hydroxybenzoic acid, **11**. The levels of compounds **8**-**11** are commonly determined by GC analysis of their trimethylsilyl ethers.



Nitrobenzene oxidation is useful for determining the amounts and relative proportions of uncondensed *p*-hydroxyphenyl and guaiacyl units in lignin. The total yield of monomeric oxidation products provides a relative measure of the extent of condensation of the lignin.

2.4.3.2 Ethanolysis

When wood is refluxed with ethanol containing hydrochloric acid the so-called "Hibberts ketones" are formed by acid-catalysed solvolysis of β-O-4 units in lignin (*59,78*) (Figure 2.13). The total yield of ethanolysis products, even where it amounts to less than 10% of the lignin, has been used as an indication of the amount of β-O-4 units present (*89,90*).

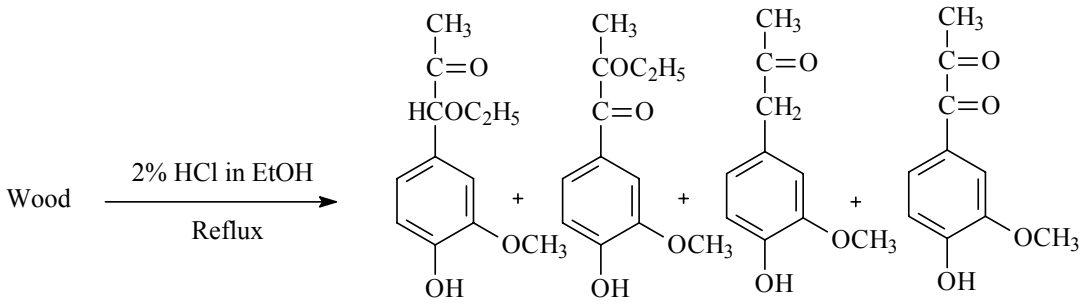


Figure 2.13 Hibberts ketones production by ethanolysis of wood.

2.4.3.3 Thioacidolysis

In recent years, thioacidolysis is proving to be one of the most widely utilized diagnostic methods in the study of lignin structure. It is routinely used to estimate the amount and composition of uncondensed β-O-4 structures (*79,80,94*).

Thioacidolysis is carried out in anhydrous dioxane using a hard Lewis acid, boron trifluoride etherate and a soft nucleophile, ethanethiol, at elevated temperature. The suggested mechanism of the cleavage of β-O-4 linkages is shown in Figure 2.14.

The initial reaction occurs through coordination of the vacant orbital on the BF_3 with benzylic oxygen (α-OH) to form an oxonium ion. This is followed by nucleophilic attack by EtSH at the activated benzylic carbon, which leads to substitution at Cα and elimination of the OH group. Substitution at Cβ, with ether bond cleavage, takes place through intramolecular attack by the introduced thioethyl group at Cα. The EtSH attacks the cyclic sulfonium intermediate, yielding a pair of *erythro* and *threo* isomers which are independent of the stereochemistry of the starting material. Substitution at Cγ then follows by a similar mechanism.

The main monomer resulting from guaiacyl β-ether structures is 1-(4-hydroxy-3-methoxyphenyl)-1,2,3-(*tris* thioethyl)propane, **12**, while *p*-hydroxyphenyl β-ethers give rise to the 1-(4-hydroxyphenyl)-1,2,3-(*tris* thioethyl)propane, **13**. These products can be monitored by GC-MS as their trimethysilyl (TMS) derivatives, and the proportions of guaiacyl and *p*-hydroxyphenyl β-O-4 structures can be determined.

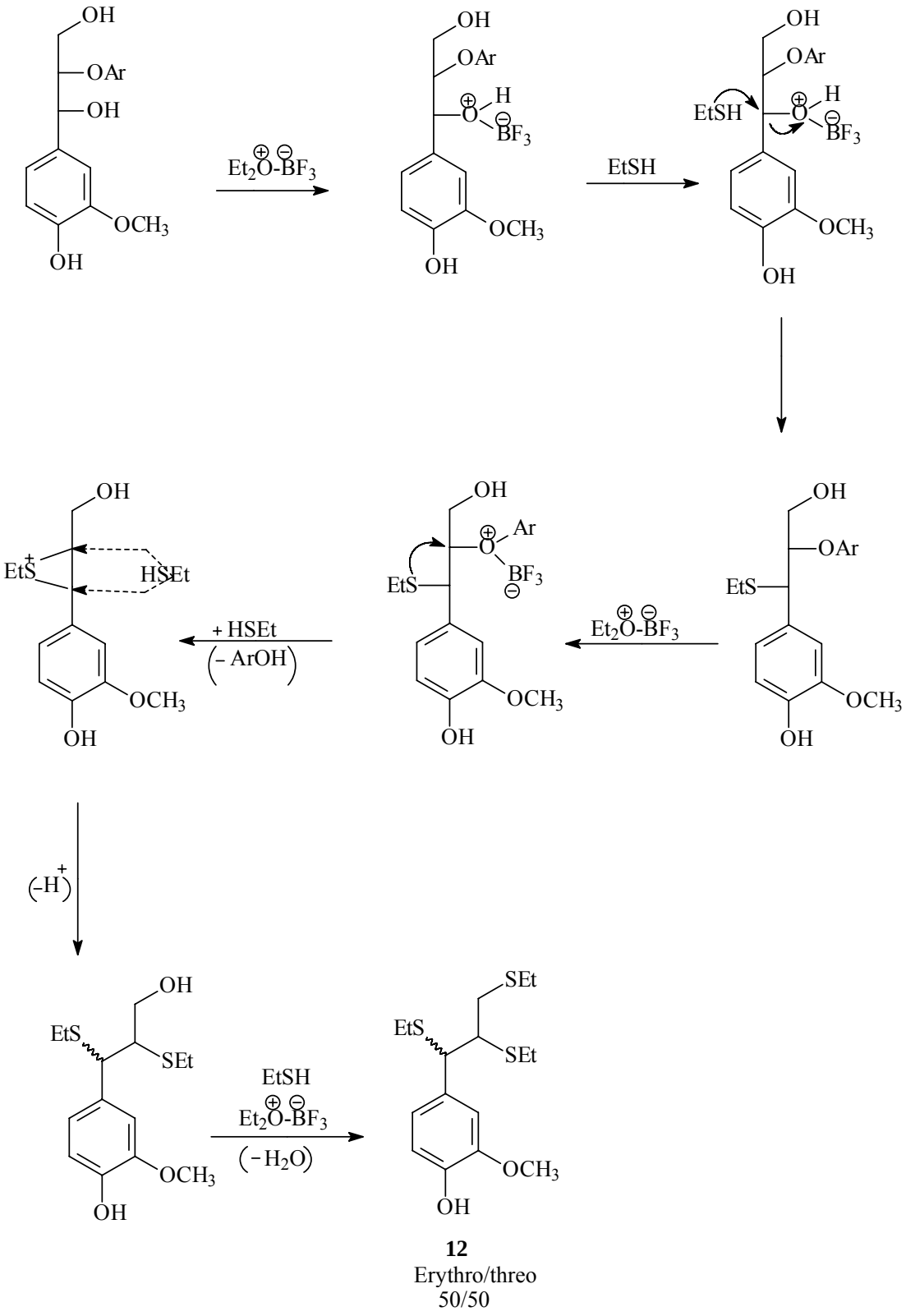
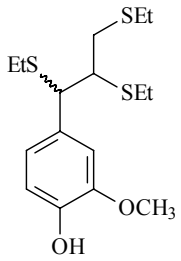
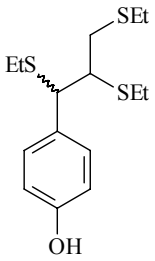


Figure 2.14 Proposed mechanism for lignin thioacidolysis (*79*).



12



13

Thioacidolysis only measures β-O-4 ether units that are phenolic or linked at C4 through an ether bond to the α- or β-position of an adjacent unit in the lignin. Other β-O-4 units, e.g. those linked to other units by a C-C bond at C5, are not converted to the monomers **12** or **13** on thioacidolysis. β-ethers that give rise to monomers **12** or **13** have been regarded as "uncondensed" β-ethers (*94*), but the term "releasable" suggested by Ralph (*95*) is used here to avoid confusion with the term "uncondensed C9 units".

Thioacidolysis can be applied to both *in situ* wood lignins and isolated lignins. It has a higher reaction yield and a far less complex mixture of monomeric products than ethanolysis. However, thioacidolysis uses malodorous ethanethiol and is therefore not desirable for many researchers to use.

Derivatisation followed by reductive cleavage (DFRC) is a more recent technique, which also measures alkyl aryl-ether bonds in lignin (*81*). However, the cleavage of aryl-ether bonds by DFRC have found to be incomplete in comparison to thioacidolysis (*96*).

2.4.3.4 Ozonolysis

Ozonolysis involves selective degradation of the aromatic nuclei in lignin by ozone. The resulting low molecular weight compounds retain the side chain stereochemistry of the original lignin (*82*). Ozonolysis results in the formation of erythronic and threonic acid from the corresponding *erythro* and *threo* forms of the β-O-4 structures in lignin (*97*) (Figure 2.15). The proportion of β-O-4 structures can be deduced from the total yield of erythronic and threonic acids.

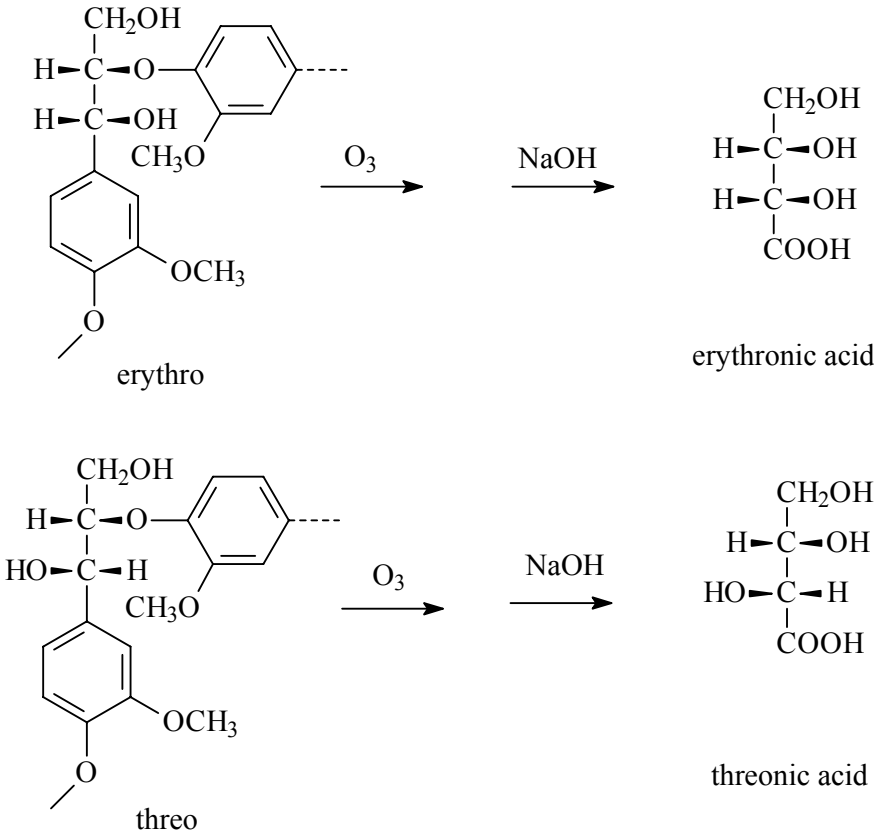


Figure 2.15 Ozonolysis: formation of two tetronic acids from side chains of β-O-4 structures.

2.4.3.5 Oxidative degradation

Oxidative degradation selectively degrades aliphatic side chains attached to phenolic aromatic moieties (*84,89,90,98*). The method involves a series of steps. Free phenolic groups are methylated or ethylated to prevent their oxidation during the process. The methylated or ethylated lignin is treated with a periodate/permanganate mixture, followed by hydrogen peroxide. The resulting mixture of acids is methylated with diazomethane and characterised by GC-MS (Figure 2.16). As discussed in Section 2.5.4.1, oxidative degradation yields a simple mixture of aromatic carboxylic acid methyl esters from free phenolic units involved in C5 and C6 condensed structures as well as uncondensed structures in lignin. This method gives no information about etherified phenolic structures.

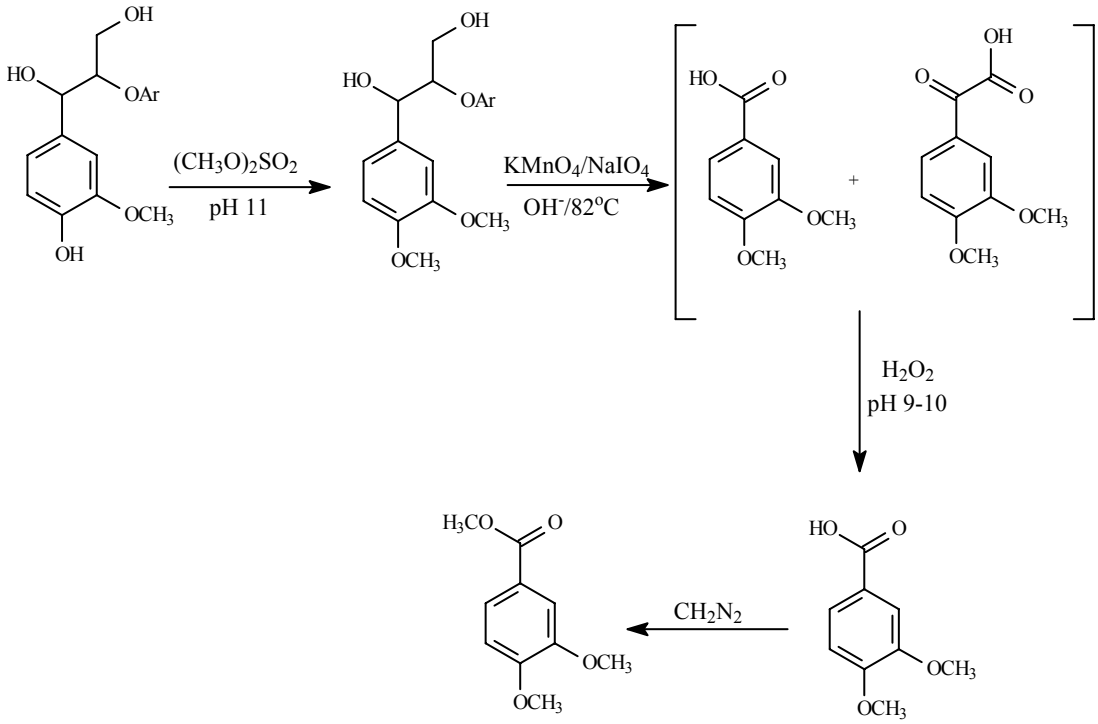


Figure 2.16 Oxidative degradation of a guaiacyl free phenolic unit (*59*).

2.4.3.6 Thioacidolysis/desulfurisation

While β-O-4 linkages in lignin are effectively cleaved by thioacidolysis, β-5, β-1, β-β, 5-5 and 4-O-5 bonds are not. Consequently, in addition to the main monomeric products, the thioacidolysis product contains a mixture of thiolated dimers, trimers and tetramers derived from the condensed structures (*85*) (Figure 2.17). Although present in low amounts, the dimers with β-5, 5-5, β-1, 4-O-5 and β-β linkages thus represent corresponding lignin inter-unit bonds referred to as condensed linkages. Due to their high molecular weight and large number of isomers, these highly thiolated dimers are difficult to determine by GC. Thioethyl groups can be removed from the side chains by Raney nickel desulfurisation, which also reduces the molecular weight of the oligomer (*85*).

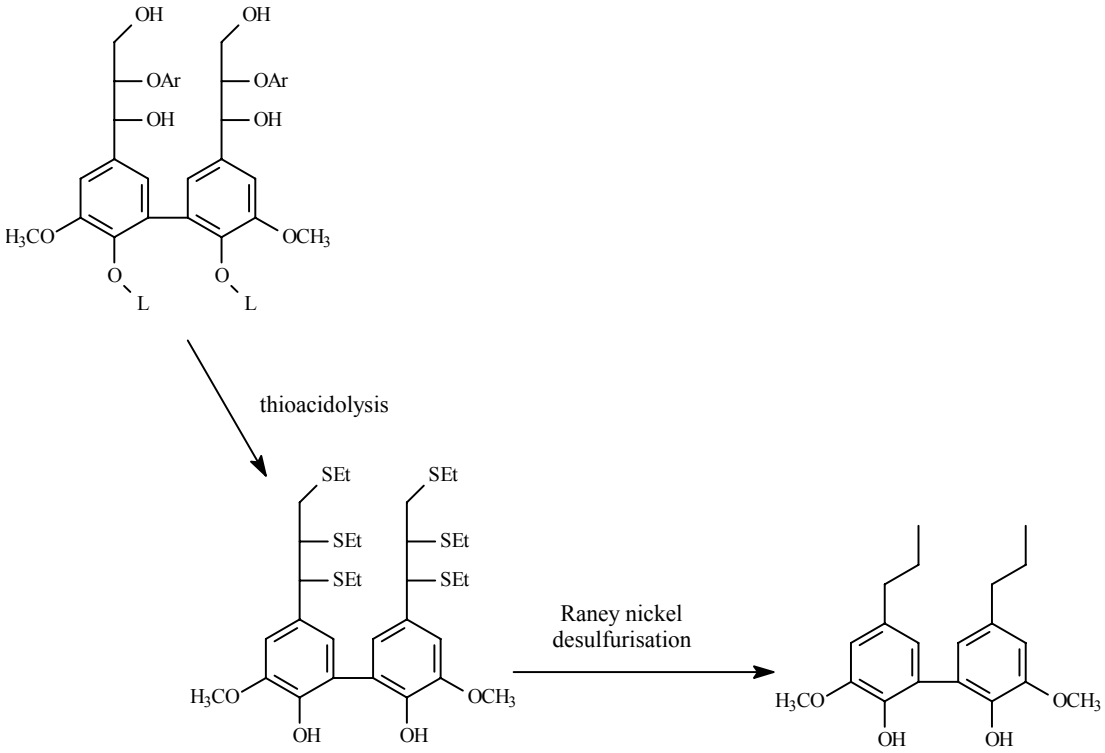


Figure 2.17 Thioacidolysis of a condensed β-ether and subsequent Raney nickel desulfurisation. L = Lignin.

2.4.3.7 Thioacidolysis/SEC

Size exclusion chromatography (SEC) is commonly used to determine the molecular weight profile of isolated lignins (*99*). The relative proportions of condensed units in lignin can be estimated from analysis of thioacidolysis products by SEC (*86,100*). The higher the proportion of C9 units in lignin linked by bonds not cleaved on thioacidolysis (β-5, β-β, 5-5, 4-O-5 etc), i.e. condensed C9 units, the greater the proportion of oligomers in the thioacidolysis product. As explained in Section 2.4.3.6, thioacidolysis products consist of monomers as well as a variety of oligomers (*86,96,100*). The proportion of GC peaks representing oligomeric structures gives an indication of the degree of condensation of the lignin.

2.4.3.8 Thioacidolysis/^{31}P NMR spectroscopy

^{31}P NMR spectroscopy has been used for quantification of hydroxyl groups in lignin. The method involves derivatisation of the free hydroxyls groups with a phosphitylating agent such as 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, followed by quantitative ^{31}P NMR spectroscopy (*101*) (Figure 2.18). The NMR

spectra contain well-defined regions for aliphatic, phenolic and carboxylic acid phosphitylated hydroxyl groups.

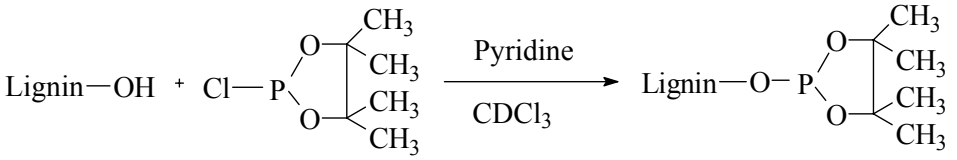


Figure 2.18 Derivatisation of hydroxyl groups of lignin with the phosphitylating reagent.

For phosphitylated phenolic moieties, the difference in the ^{31}P chemical shift is predominantly due to the nature of the groups attached in the *ortho*-positions relative to the C4 phenolic group, i.e. those in the C3 and C5 positions in the aromatic ring (*102*). Substituents at C1, 2 and 6 have little effect. This makes it possible to distinguish between uncondensed guiaiacyl units, with a proton at C5, and condensed guaiacyl units, linked at C5 to another unit via an ether or C-C linkage. Uncondensed *p*-hydroxyphenyl units in lignin can be determined in the same way (Figure 2.19) (*101*). This method only provides information on free phenolic C9 units in isolated lignins.

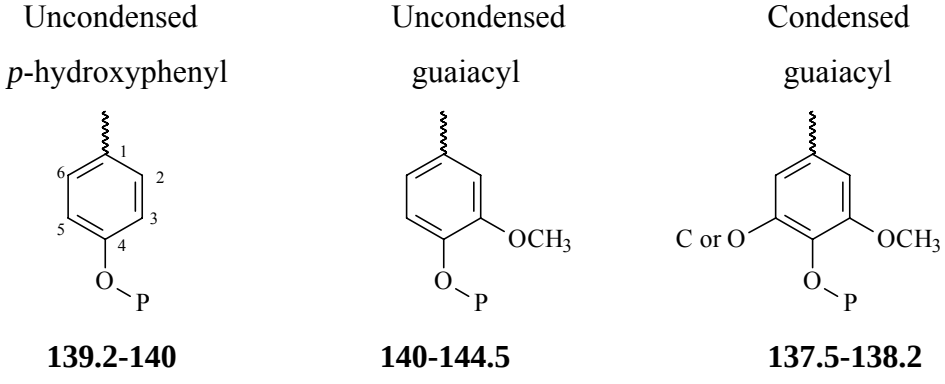


Figure 2.19 ^{31}P NMR Chemical shift ranges of common lignin structures (ppm).

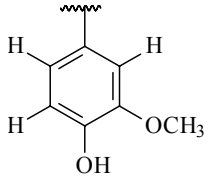
Application of ^{31}P NMR analysis to thioacidolysis products provides information on phenolic and etherified C9 units in the lignin, because thioacidolysis cleaves many of the ether inter-unit bonds to generate new phenolic C9 units (*103*). Smit *et al*. (*87,103*) used a combination of thioacidolysis and quantitative ^{31}P NMR

spectroscopy to measure the total amount of condensed and uncondensed phenolic units in whole lignin. This procedure allows quantification of three quarters of the C9 units in *in situ* lignin. Thioacidolysis/^{31}P NMR spectroscopy is the first method to be developed for simultaneous and direct determination of the proportion of condensed and uncondensed G units in *in situ* lignin. Following similar principles, DFRC coupled with quantitative ^{31}P NMR spectroscopy has been used for analysis of condensed structures in isolated lignins (*104*).

While thioacidolysis/^{31}P NMR spectroscopy allows quantification of C5-condensed units involving 5-5, 4-O-5 and β-5 inter-unit linkages, it does not reveal any information about β-β and β-1 inter-unit linkages because they do not involve the C5 position. The method is sensitive only to chemical shift differences brought out by *ortho*-substitution to the phenolic hydroxyl group.

2.4.3.9 ^{1}H NMR spectroscopy

The degree of condensation of gymnosperm lignin preparations (MWL) have been estimated by ^{1}H NMR spectroscopy (*88-90*). Aromatic protons appear in the region ~6.35–7.9 ppm. As this region is relatively free from interfering protons, it is possible to estimate the number of aromatic protons per guaiacyl C9 unit. An uncondensed guaiacyl unit, **14**, contains three aromatic protons, but a guaiacyl unit condensed at C5, **15**, contains only two aromatic protons. Subtraction of the value estimated for aromatic protons from the maximum of 3 per guaiacyl unit allows the degree of condensation to be calculated. Condensed structures involving C2 and C6 can also be estimated by this method.



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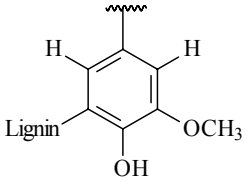

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2.4.3.10 Quantitative ^{13}C NMR spectroscopy

^{13}C NMR spectroscopic methods can be used to estimate the degree of condensation in isolated lignin samples (*91-93*). For an uncondensed guaiacyl moiety there are three aromatic C-H carbons. A guaiacyl nucleus in a condensed structure will have

less than 3 aromatic C-H carbons per guaiacyl unit. So the difference between 3 and the integral corresponding to aromatic C-H carbons gives the degree of condensation (*92*). It should be noted that condensed structures involving C2, C5 and C6 linkages can also be estimated by this method.

2.4.3.11 Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS)

Pyrolysis is the transformation of a non-volatile compound into a volatile degradation mixture by heating in the absence of oxygen (*105-108*). During analytical pyrolysis, the lignocellulosic material is rapidly heated to temperatures around 400-600°C and the propanoid side-chain structures in lignin are either cleaved completely or shortened to one or two carbons. These degradation products can be separated by GC and identified by MS (Figure 2.20 and Table 2.6). Detailed information about the degradation products has been published, and this helps with interpretation of the pyrograms (*107,109,110*). The nature of the products formed depends on the structure of the lignin, therefore py-GC-MS can be used for determining the ratio between H and G units in lignin (*108*). Pyrolysis-GC-MS has also been used to fingerprint and identify residual lignins (*107*).

The py-GC-MS method is only semi-quantitative. Simple sample preparation (drying and milling of samples), rapid analysis times, and small sample size (micrograms) are key features. Use of an internal standard to improve accuracy has been suggested (*111*).

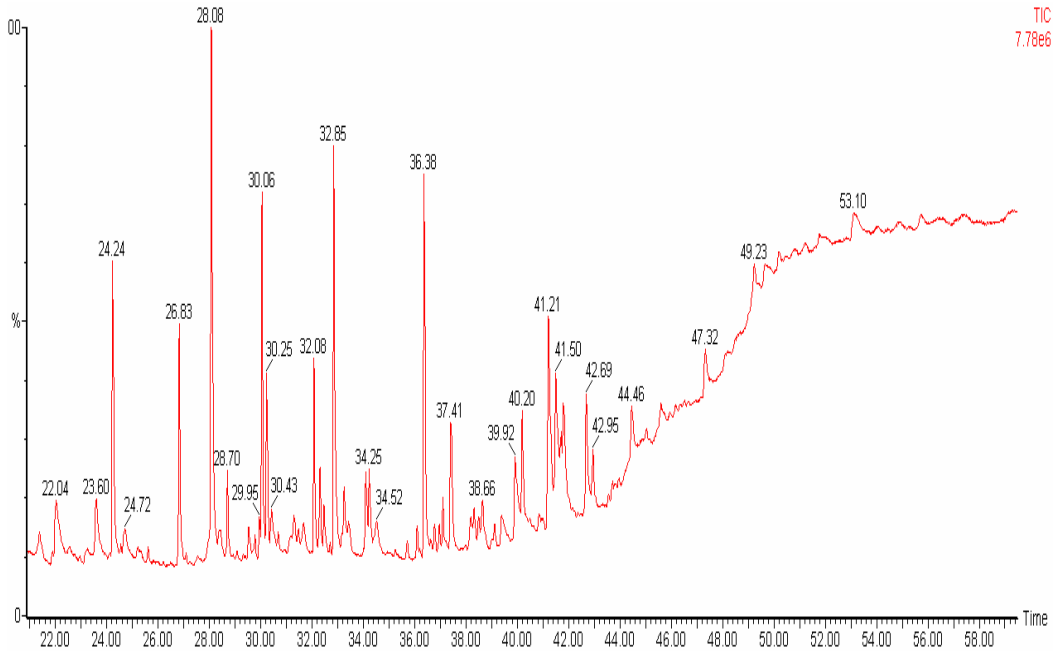


Figure 2.20 A typical pyrogram derived from *Pinus radiata* wood (only lignin derived peaks are shown).

Table 2.6 Main lignin pyrolysis products from *Pinus radiata* wood. Degradation products are classified as guaiacyl (G) and *p*-hydroxyphenyl (H).

Retention time (min)	Mass	Product	Origin
24.2	124	2-Methoxy phenol (guaiacol)	G
26.8	138	4-Methyl-guaiacol	G
28.1	94, 107	Phenol and 2-methyl phenol	H
28.7	137	4-Ethyl guaiacol	G
30.1	108	4-Methyl phenol	H
30.3	108	3-Methyl phenol	H
32.1	164	Eugenol	G
32.3	121	4-Ethyl phenol	H
32.9	135	4-Vinyl guaiacol	G
34.3	164	Isoeugenol (*cis*)	G
36.4	164	Isoeugenol (*trans*)	G
37.4	120	Vinyl phenol	H
40.2	134	Propenyl phenol	H
41.2	151	Vanillin	G
41.5	137	Homovanillin	G
42.7	151	Acetovanillone	G
44.5	137	Coniferyl alcohol	G
49.2	137	Dihydroconiferyl alcohol	G
53.1	178	Coniferaldehyde	G

2.5 Compression wood lignin

Extensive investigations of differences between the lignin structure of CW and normal wood have been described (*48,54,59,62,89-91,100,112-120*). It is generally agreed that, CW lignin has:

- A higher proportion of *p*-hydroxyphenyl (H) units
- Fewer β-O-4 linkages
- A higher content of free phenolic C9 units
- An increased proportion of condensed-type linkages particularly 5-5 type linkages.

2.5.1 p-Hydroxyphenyl (H) units

The high proportion of H units in CW lignin was discovered by Bland (*48*) using the nitrobenzene oxidation. It was subsequently confirmed by other authors (*62,89,90,112,119-121*). The CW produced higher levels of *p*-hydroxybenzaldehyde, **10**, and lower levels of vanillin, **8**, than normal wood (Table 2.7).

Table 2.7 Lignin content (wt % of oven dry wood) and nitrobenzene oxidation yield (wt % of lignin) of normal wood (NW) and compression wood.

	NW				CW			
	Lignin content	Yield			Lignin content	Yield		
		10	**8**	Total		**10**	**8**	Total
Pinus radiata (*48*)	25.8	Nil	20.2	20.2	34.4	0.19	17.7	17.9
Abies sachalinensis (*112*)	28.3	0.5	17.1	17.6	35.3	1.9	12.1	14.0
Pseudotsuga menziesii (*62*)	-	2.1	15.5	17.6	-	4.2	9.8	14.0
Larix leptolesis (*90*)	28.1	0.8	22.6	23.4	39.3	2.6	15.1	17.7
Pinus tedea (*119*)	29.1	0.2	26.1	26.3	36.6	2.3	19.9	22.2

Levels of H units reported in CW diverge considerably. According to Latif (*62*) CW lignin of *Pseudotsuga menziesii* contained 30% of H units, compared with 12% in normal wood lignin. Erickson *et al*. (*98*) reported 20% of H units in *Pinus mugo*, which was about 4 times higher than the proportion in normal wood. Smit *et al*. (*87*)

found that H units represented approximately 8% of the C9 units in mild CW latewood, compared with approximately 2% in normal wood. Fukushima and Terashima (*121*) reported approximately 18% H units in *Pinus thunbergii* CW. A more recent study has reported 30% of H units in spruce CW lignin (*100*).

The total nitrobenzene oxidation yield from CW was lower, indicating that there are fewer uncondensed linkages in CW lignin than in normal wood lignin (Table 2.7).

The methoxyl content of CW lignin is lower than that of normal wood (Table 2.8). This would be expected from higher levels of H units.

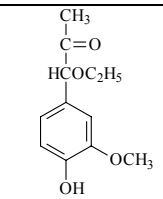
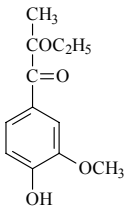
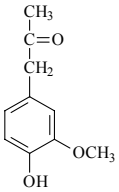
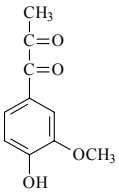
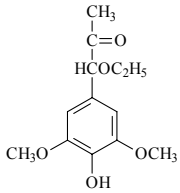
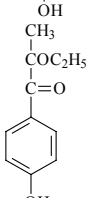
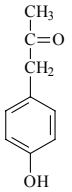
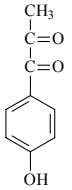
Table 2.8 Methoxyl content of normal wood and compression wood (%).

Species	NW	CW
Pinus radiata (*48*)	14.1	12.6
Abies sachalinensis (*112*)	15.3	13.2
Larix leptolesis (*90*)	15.2	12.6

2.5.2 β-O-4 linkages

The overall lower yield of Hibberts ketones (products **16**-**23**) produced by ethanolysis of CW (10.4% *vs*. 12.4% for OW) indicates that there are fewer β-O-4 units in CW than in normal wood (*89*,*90*) (Table 2.9). Products **21**-**23** were not formed from normal wood, and this provided further rationale for the presence of higher levels of H units in CW (*89*).

Table 2.9 Main products of ethanolysis and their yields from normal wood and compression wood of *Abies sachalinensis* (%)(*89*).

Product	Compound No.	NW	CW
	**16**	1.24	0.99
	**17**	5.72	2.88
	**18**	2.06	1.28
	**19**	3.12	3.34
	**20**	0.24	0.15
	**21**	-	1.0
	**22**	-	0.29
	**23**	-	0.37

Using ozonolysis, Yeh *et al*. (*120*) showed that CW has fewer β-O-4 linkages and a higher *erytho/threo* ratio (1.23) than normal wood, where the ratio is approximately

1.00. This indicates that the lower proportion of β-O-4 linkages in CW lignin is the result of a decrease in *threo* isomers rather than *erythro* isomers.

The yields of releasable β-ethers determined by thioacidolysis of CW and normal wood are given in Table 2.10. The higher yield of H units from thioacidolysis of CW agrees with results obtained by other techniques such as nitrobenzene oxidation and ethanolysis.

Table 2.10 Thioacidolysis yields of releasable β-ethers from compression wood and normal wood (μmol/g lignin).

		12	**13**	Total	H/G
Pinus pinaster (*80,94*)	NW	992	25	1017	0.03
	CW	934	207	1141	0.22
Picea abies (*100*)	NW	1682	-	1682[1]	-
	CW	975	416	1391[1]	0.43

[1] After preswelling of the sample.

There is some inconsistency in results reported for total releasable β-ethers. Önnerud and Gellerstedt (*100*) reported lower yields of releasable β-ethers from CW than from normal wood, which suggests that fewer β-O-4 units are present. Lapierre and co-workers (*80,94*) did not find significant differences between CW and normal wood thioacidolysis yield.

2.5.3 Free phenolic C9 units

Compression wood lignin has a slightly higher free phenolic content than from normal wood (*90*). Yeh *et al*. (*120*) using ^{13}C NMR spectra reported a slightly higher phenolic content (27 *vs.* 26 OH/C9 unit) in cellulolytic enzyme lignin obtained from CW compared with that derived from opposite wood.

Lapierre *et al*. (*122,123*) analysed CW lignin from *Pinus pinaster* by thioacidolysis of diazomethane-methylated *in situ* lignin and MWL (the principles of this method are discussed in Chapter 7). They found that 90% of the H units were phenolic in both the *in situ* lignin and MWL. These results were confirmed by Smit *et al*. (*87*),

who reported that 85–90% of H units in MWL isolated from *Pinus radiata* normal wood and CW were phenolic.

Most H units exist as free phenolic entities in lignin dehydrogenation polymers (DHP) (*69*). In H-G (*p*-hydroxyphenyl-guaiacyl) and H-G-S (*p*-hydroxyphenyl-guaiacyl-syringyl) DHPs produced by bulk and endwise polymerisation, 70-80% of the β-O-4 linked H units have free phenolic groups (terminal units). This suggests that H units are incorporated into β-O-4 structures essentially as terminal units in both synthetic DHP and native lignin.

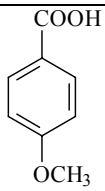
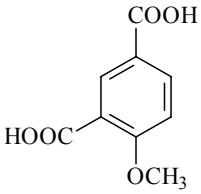
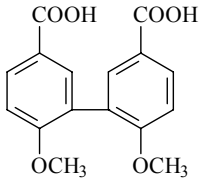
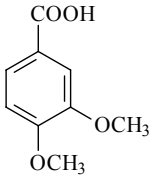
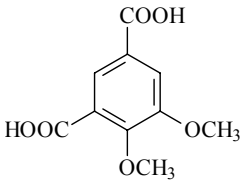
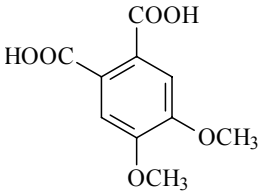
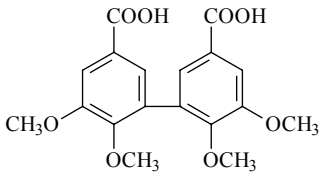
2.5.4 Condensed structures

2.5.4.1 Oxidative degradation

A comparison of the yields of the main carboxylic acids obtained from permanganate oxidation of CW and normal wood of *Larix leptolesis* is shown in Table 2.11 (*90*). Compression wood gave higher yields of anisic acid, **24**, isohemipinic acid, **28,** 4-methoxyisophthalic acid, **25**, as well as biphenyl carboxylic acid **26** and **30**. Normal wood yielded greater amounts of veratric acid, **27,** metahemipinic acid, **29**. The total yield of permanganate oxidation was 16.6% of Klason lignin in CW and 14.5% in normal wood. These results showed that in comparison with normal wood of *Larix leptolesis* CW has:

- A higher proportion of H units, indicated by higher yields of **24-26**.
- More condensed-type structures, indicated by higher yields of **25, 26, 28** and **30**.
- More carbon-carbon (5-5) linkages indicated by higher yields of **26** and **30**.

Table 2.11 Degradation products from oxidative degradation of lignin from *Larix leptolesis* (% of Klason lignin) (*90*).

Origin	Product	Compound No.	NW	CW
Uncondensed H		**24**	0.91	2.47
H condensed 5		**25**	0.11	0.27
H condensed 5-5		**26**	0.10	0.14
Uncondensed G		**27**	12.14	10.17
G condensed 5		**28**	1.07	2.41
G condensed 6		**29**	0.20	0.12
G condensed 5-5		**30**	0.10	0.14

Erickson *et al*. (*98*) reported that small amounts of the dimers shown in Figure 2.21 were formed during permanganate oxidation of CW in addition to the structures shown above. The structure of these dimers indicates that CW lignin is formed by copolymerisation of *p*-coumaryl alcohol and coniferyl alcohol i.e. CW is a G-H lignin.

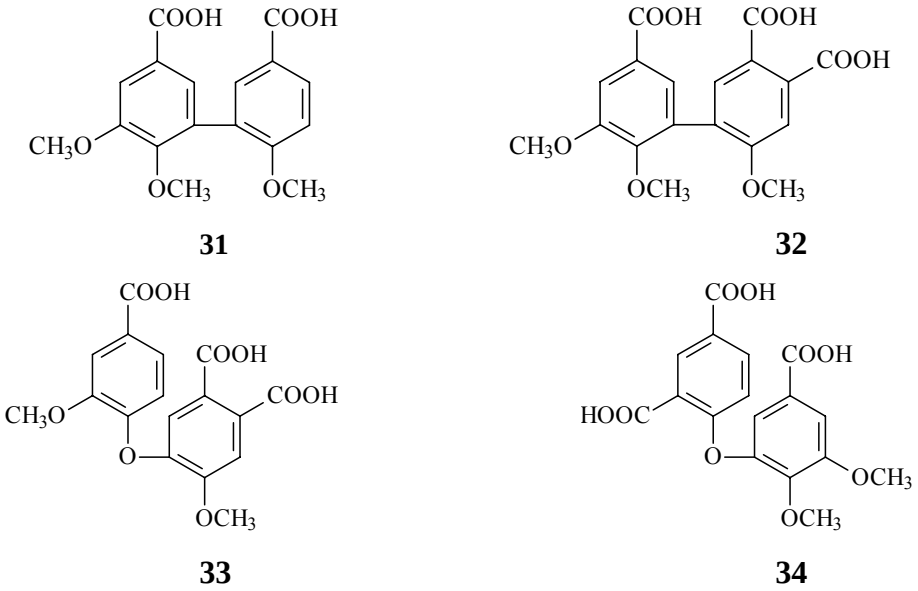


Figure 2.21 Dimeric structures formed by copolymerisation of *p*-coumaryl alcohol and coniferyl alcohol derived from CW of *Pinus mugo* by permanganate oxidation (*98*).

It is important to note that all products of permanganate oxidation are derived from structures that exist as free phenolic units in the original lignin and that the method reveals little information about etherified lignin components. Free phenolic units account for only 10-12% of the total C9 units in lignin (*124*).

2.5.4.2 Thioacidolysis/Raney nickel desulfurisation

Recently, G-G and G-H dimers (β-1, β-5, β-6, and 4-0-5) have been identified by thioacidolysis combined with Raney nickel desulfurisation of spruce CW and normal wood (*125*). Many of the G-G and G-H structures found in normal wood were also present in CW. However, dimers and trimers derived from H units alone were only found in CW.

Saito and Fukushima (*126*) attempted to quantify G-G, G-H and H-H dimers in 5-5, β-5, 4-O-5, β-1 and β-β type linkages in *Pinus thunbergii* CW and normal wood. Dimer analysis showed that the relative distribution of condensed linkages of G-G, G-H and H-H dimers were not significantly different between normal wood and CW (Table 2.12). The H-H dimers were detected in CW only, in relatively small proportions (1% compared to 83.4% for G-G). 5-5 and β-5 structures were prominent in H-H, G-H and G-G dimers in CW and G-H and G-G dimers in normal wood.

Table 2.12 Relative distribution (mol%) of dimers resulting from thioacidolysis and subsequent desulfurisation of normal and compression wood (*126*).

Dimer	NW	CW
5-5	36.1	37.1
β-5	27.0	24.4
4-O-5	7.8	11.5
β-1	26.0	23.6
β-β	3.1	3.3
Total (%)		
G-G	99.3	83.4
G-H	0.7	15.6
H-H	-	1.0

Saito and Fukushima (*126*) compared the frequency of dimers obtained in their study with the relative frequencies of bonding patterns for dehydrogenation polymer (DHP) dimers obtained by Jacquet *et al*. (*69*) through bulk polymerisation and end-wise polymerisation. They concluded that dimers obtained from both CW and normal wood were the result of end-wise polymerisation. This conflicts with the theory that CW is a bulk polymer (Section 2.3.3.2). Nevertheless, it should be noted that the process of polymerisation *in vitro* may differ from polymerisation in the cell wall.

2.5.4.3 Thioacidolysis/SEC

Suckling *et al*. (*86*) have studied size exclusion elution profiles of the thioacidolysis products of lignin from *Pinus radiata* CW and normal wood lignin from the same tree. A higher proportion of condensed units in the CW was indicated by the greater area under the high molecular weight (early eluting) peaks (Figure 2.22). Monomer peaks have been normalised to the highest peak. This observation has been further confirmed by Önnerud and Gellerstedt (*100*).

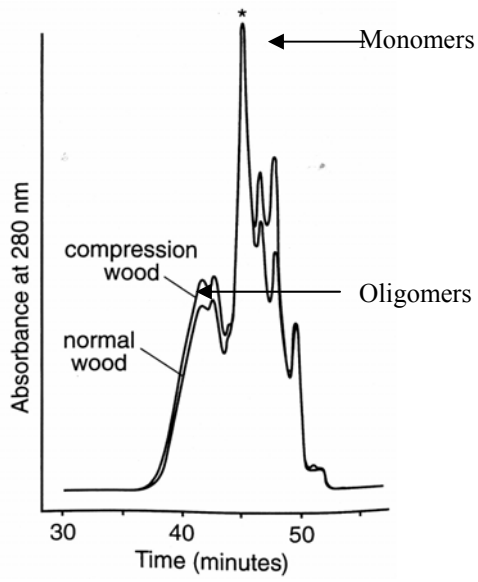


Figure 2.22 Size exclusion chromatograms of thioacidolysis products from normal and CW-rich *Pinus radiata* increment cores (*86*). * - normalized to highest peak.

2.5.4.4 Thioacidolysis/ ^{31}P NMR spectroscopy

Using thioacidolysis/^{31}P NMR spectroscopy it was found that CW contains a smaller proportion of uncondensed G units and a higher proportion of uncondensed H units than normal wood (*87*). On the other, hand there was no significant difference between *Pinus radiata* CW and normal wood in the proportion of guaiacyl C5 condensed structures (*87*) (Table 2.13). This result agrees with the Saito and Fukushima's findings discussed in Section 2.5.4.2.

Table 2.13 Thioacidolysis/^{31}P NMR products from *Pinus radiata* compression wood and normal wood (C9 units mmol/g lignin) (*87*).

	NW	CW
Condensed G	1.58	1.53
Uncondensed G	2.73	2.34
Uncondensed H	0.09	0.34
Total phenolics	4.40	4.23
% condensed	36	36

2.5.4.5 ^{1}H and ^{13}C NMR spectroscopy

The proportion of condensed units in isolated lignin samples as estimated by NMR methods is summarised in Table 2.14. ^{1}H NMR analysis of acetylated MWLs suggests that CW lignin has a higher content of condensed units than normal wood (*89,90,112*). As discussed earlier, this method measures condensation at the C2, C5 and C6 positions in the aromatic ring.

Table 2.14 Degree of condensation (%) of MWL from five tree species as measured by NMR spectroscopy.

Species	Method	NW	CW
Pinus radiata (*88*)	^{1}H NMR	42	-
Abies sachalinensis (*89,112*)	^{1}H NMR	48	79
Larix leptolesis (*90*)	^{1}H NMR	56	84
Picea abies (*92*)	^{13}C NMR	38	-
Abies fraseri (Purch) Poir (*93*)	^{13}C NMR	29	-

Nimz *et al*. (*91*) compared the ^{13}C NMR spectrum of a CW MWL sample of *Larix leptolesis* used in an earlier study (*90*) with that of normal spruce MWL. They found greater representation of peaks associated with *p*-hydroxyphenyl residues in the CW lignin. In the aliphatic region (δ50–90 ppm), peaks characteristic of β-O-4 linkages were less pronounced in the CW spectrum.

2.5.4.6 Summary

Earlier workers considered that CW lignin was a condensed-type lignin formed through bulk polymerisation and containing a higher percentage of *p*-coumaryl alcohol moieties than normal wood lignin (*54*). From the above discussion, it seems clear that the more recent literature confirms the presence of higher proportions of *p*-coumaryl alcohol moieties in CW lignin. It also suggests that the degree of condensation in CW lignin is similar to that in normal wood lignin (*87,126*). It should be noted that the various techniques used reveal different aspects of CW lignin. There is no universal technique whereby the level of condensation can be measured. The structure of CW lignin is far from being fully described, but the

emergence of modern technologies will undoubtedly contribute to our understanding of CW lignin.

2.6 Variation of lignin distribution in cell walls of normal wood

Detailed reviews of the content and composition of lignin in specific cell wall layers have been written by Terashima *et al*. (*127*) and Donaldson (*128*).

Examination of *in situ* wood sections by UV microscopy has shown that although the highest lignin concentration in softwoods (70%) occurs in the middle lamella, most of the lignin (90%) is found in the secondary wall (*129,130*). This is because the secondary layer makes up a much larger proportion of the cell wall (*128,130,131*). The lignin content of individual cell wall layers has also been studied by isolating middle lamella and secondary wall-enriched fractions from finely-ground cell wall fragments or from mechanical pulps (*100,132-135*). In the middle lamella-rich fraction the lignin content was as high as 60%. The remaining fiber, composed mainly of secondary wall material, had a lignin content very similar to that of the whole wood sample (28%). Lignin distribution as assessed by microscopy seems to agree quite well with that determined by analysis of isolated cell wall fractions.

There are reported variations in the structure of middle lamella and secondary wall lignins. For example, middle lamella lignin was found to have fewer β-O-4 structures than secondary wall lignin (*100,134-136*), suggesting that middle lamella is more condensed. Adams (*137*) found no difference in the proportion of releasable β-O-4 units in fibres (secondary wall rich) and fines (middle lamella rich) separated from *Pinus radiata* thermomechanical pulp. Compared with secondary wall lignin, middle lamella lignin was found to be richer in β-β and 5-5 structures, suggesting that middle lamella lignin was more condensed than secondary wall lignin (*134*). Terashima and Fukushima also reported that condensed guaiacyl structures were formed preferentially during the earlier stages of lignification of the middle lamella region (*67,127,138*). According to Christiernin *et al*. (*139*) middle lamella has a slightly higher proportion of β-5, β-1, β-5 and 5-5-O-4 structures than secondary wall.

While several studies (*132,135*) have reported that the content of phenolic hydroxyl groups is twice as great in secondary wall lignin as in middle lamella lignin, others (*134*) have recorded a smaller difference. There are also reports that middle lamella lignin has a lower methoxyl content and a higher carbonyl content than secondary wall lignin (*135*).

Whiting and Goring (*133*) claimed that the lower methoxyl content in middle lamella lignin was due to a higher *p*-hydroxyphenyl content. Westermark (*136*), on the other hand suggested the presence of *p*-hydroxyphenyl units was due to contamination with CW lignin since she found no indication of *p*-hydroxyphenyl units in middle lamella isolated by a sieving technique. Fukushima and Terashima (*121*) studied the lignification process in xylem by microautoradiography after administering radiolabelled lignin precursors. They found high levels of *p*-hydroxyphenyl lignin in the middle lamella of *Pinus thunbergii* tracheids and speculated that *p*-hydroxyphenyl units in compound middle lamella may be highly condensed, making determination by conventional degradative means such as nitrobenzene oxidation more difficult (*127*). Christiernin *et al*. (*139*), comparing MWL of spruce (supposedly derived from secondary wall), with MWL prepared from old spruce in which the secondary wall had been lost through decay, reported very low levels of *p*-hydroxyphenyl units in this middle lamella-derived lignin, a result which agreed with Westermark's (*136*) observation.

2.7 Compression wood polysaccharides

2.7.1 Cellulose

Cellulose, which makes up about one third of the total weight of wood, is a linear homopolymer of (1→4)-β-D-glucopyranose residues (Figure 2.23). The degree of polymerisation (DP) reported for native cellulose ranges between 3,000 and 10,000 (*140,141*), although the general consensus is that a DP of 10,000 is typical of undegraded cellulose in the normal wood of conifers. A strong tendency for intra- and intermolecular hydrogen bonding causes bundles of cellulose molecules to aggregate into microfibrils, which form either highly ordered (crystalline) or less ordered regions (*142*).

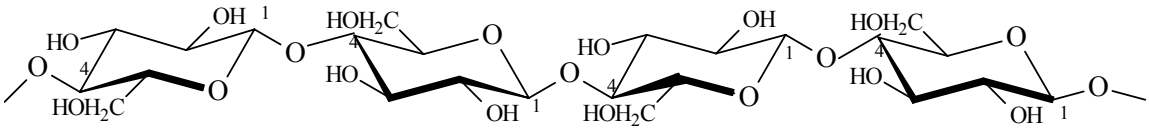


Figure 2.23 Partial structure of cellulose (*4*).

Normal softwood comprises 40-45% of cellulose; CW only 30-35%. Current knowledge about cellulose in CW is limited, because it has not been isolated. Tanaka *et al*. (*141*) reported that celluloses in *Pinus densiflora* opposite wood and CW had the same DP (~ 3,000). This value is at the low end of the range reported for softwood celluloses (*140*).

2.7.1.1 Cellulose microfibril angle (MFA)

The microfibril angle is the angle at which cellulose microfibrils are oriented to the cell axis. In the S2 layer of normal wood of conifers the MFA is approximately 15°, whereas in CW it is as much as 45° (*17*). The wider MFA may be one of the causes of greater longitudinal shrinkage in CW (*143*), which is a highly undesirable property in utilisation of wood for timber.

2.7.1.2 Degree of crystallinity in cellulose

Crystallinity and the size and orientation of crystallites in CW have been studied by various workers (*52,141,144,145*). Tanaka *et al*. (*141*) reported a crystallinity of 0.45-0.50 in *P*. *densiflora* CW compared with approximately 0.50 in normal wood and 0.50-0.60 in opposite wood. They pointed out in CW, which has higher compressive strength and lower tensile strength, cellulose has shorter crystallites and a lower degree of crystallinity. In contrast opposite wood, with a higher tensile strength, has longer cellulose crystallites and a higher degree of crystallinity. Parham (*144*) found no detectable differences in terms of crystallinity between CW and normal wood. Using ^{13}C CPMAS (Cross Polarisation Magic Angle Spinning) NMR technique, Newman (*146*), recently reported crystallinity values of 0.501 for CW and 0.507 for opposite wood. In summary, it can be concluded that there are only small, if any differences in the cellulose crystallinity of CW and normal wood.

2.7.2 Galactan

Compression wood has a much higher proportion of galactan, 10% or more, than normal wood (*54*). Galactan occurs only in the tracheids and is absent from the ray cells of CW (*147*). Most of the galactan is located in the primary cell wall and in the outer portion of the secondary layer (*148*,*149*).

It has been suggested that at least a portion of the galactan in the cell wall is linked to lignin and forms lignin-carbohydrate complexes (LCC). Lignin-carbohydrate complexes isolated from CW of *Pinus* species were found to have a higher galactose content than those from normal wood (*120*,*150*,*151*). The main polysaccharide combined with lignin in CW LCC is therefore galactan. In normal wood LCC glucomannan, is the main lignin-carrier (*152*). Mukoyoshi *et al*. (*150*) extracted LCC from red pine CW and showed by methylation analysis that galactose linked to the lignin originated from galactan consisting of (1→4)-linked-D-galactopyranose residues, which accounted for 30-40% of the neutral sugars. Koshijima and Watanabe have presented more information about CW LCC (*152*).

Compression wood galactan has been isolated from *Picea abies* (Norway spruce) (*153*), *P. rubens* (Red spruce) (*149*) and *Larix laricina* (Tamarack) (*154*). Structural analysis showed that the galactan of Tamarak consisted of 200-300 1,4-β-D-galactopyranose units (*154*). Approximately 5% of galactose residues carried a single, terminal β-D-galacturonic acid residue attached at C6 (Figure 2.24). A smaller number of glucuronic acid residues were sometimes present.

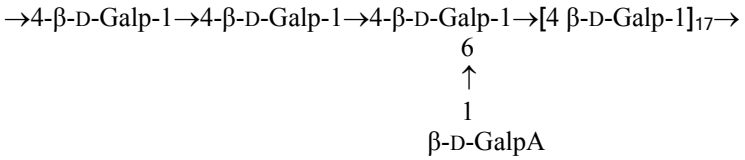


Figure 2.24 Partial structure of a CW galactan (*54*).

Its function in the living tree is, as yet, unknown. It may have a role in the formation of the secondary wall before lignin has been deposited (*149*).

2.7.3 (1→3)-β-D-Glucan (Laricinan)

Laricinan in CW was first isolated from *Larix laricina* (*155*). A similar polysaccharide has been isolated from other species and it is likely that this (1→3)-β-D-glucan occurs in most CW. The levels of laricinan in CW are generally less than 3% of the total wood weight. Only trace amounts are found in normal wood. Laricinan has a DP of 200-300 and is composed mainly of (1→3)-β-D-glucopyranose residues. There are also some (1→4) linkages and possibly D-glucuronic acid side chains although it is not clear how the latter are attached (*46*) (Figure 2.25). Laricinan has been found in the helical cavities of S2 and a role in the compressive force exerted in CW has been suggested (*156*).

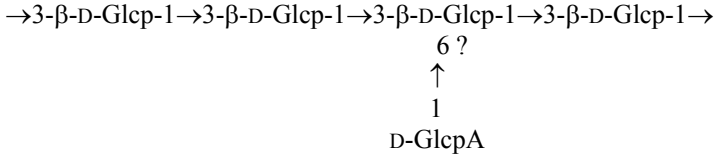


Figure 2.25 Partial structure of laricinan (*54*).

2.7.4 Galactoglucomannan

Compression wood contains about 9% of *O*-acetyl-galactoglucomannan. This is about half the amount present in normal wood (*54*). Galactoglucomannans isolated from CW of *Larix laricina* (*157*) and *Abies balsamea* (*158*) had galactose:glucose:mannose ratios of 0.2:1:2.5 and 0.5:1:3 respectively. This polysaccharide composition appears to be identical with that of normal wood galactoglucomannan. Galactoglucomannan has a main chain of (1→4)-β-D-glucopyranose and β-D-mannopyranose units with a side chain of α-D-galactopyranose attached at C6 (*157*) (Figure 2.26). The low galactoglucomannan content of CW is reflected in the relative mannose content of CW and normal wood (Table 2.2). The acetyl content of CW is about 60% of the acetyl content of normal wood (Table 2.2). It is not known whether the galactoglucomannans are partly acetylated in CW, as they are in normal wood.

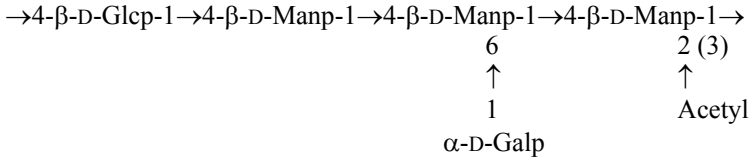


Figure 2.26 Partial structure of galactoglucomannan (*54*).

2.7.5 Arabino-4-O-methylglucuronoxylans

The arabino-4-*O*-methylglucuronoxylan content of CW and normal wood is almost identical. This polysaccharide has been isolated from CW of *Larix laricina* (*159*) and has a backbone of (1→4)-β-D-xylopyranose residues carrying side chains of 4-*O*-methyl-α-D-glucuronic acid residues attached to C2, and α-L-arabinofuranose units linked to C3 (Figure 2.27). This structure is similar to that of the xylan in normal wood of *Larix laricina*, but in CW it has only half as many arabinose sidechains.

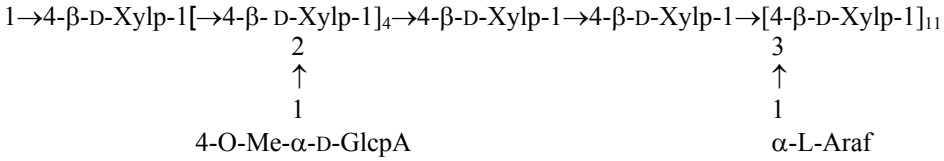


Figure 2.27 Partial structure of a xylan (*54*).

2.7.6 Arabinogalactan

Arabinogalactans are highly branched polysaccharides which are formed with xyloglucan, pectin and cellulose in the primary cell wall of plants (*54*). Arabinogalactans present in normal wood and CW of *Larix laricina* appears to have similar structures and molecular properties (*160*) (Figure 2.28). The main chain of (1→3)-β-D-galactopyranose residues, all carry side chains attached to C6. These side chains are β-L-galactopyranose, several (1→6)-β-D-galactopyranose units, and a terminal β-L-arabinopyranose linked to C3 of an α-L-arabinofuranose residue. Less frequently, β-D-glucuronic acid residues may be present. The much-branched structure is responsible for the high water solubility of this hemicellulose.

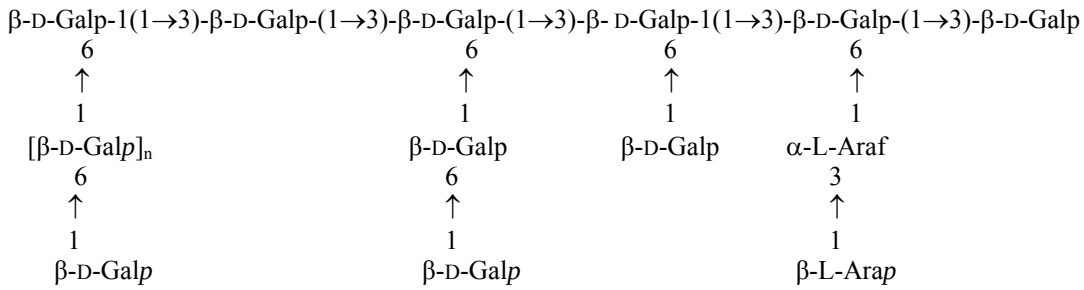


Figure 2.28 Simplified partial structure of arabinogalactan (*54*). n = unknown.

2.8 Extractives

Extractives are compounds that can be extracted from the wood by water or by neutral organic solvents such as methanol, ethanol or dichloromethane (*161*). Softwoods usually contain 1-5% extractives (on dry wood weight basis).

A comparison of the extractives from CW and normal wood from two different species is shown in Table 2.15 (*90*). The amounts vary with type of solvent used. Compression wood of both species contains higher amounts of total extractives than normal wood.

Table 2.15 Extractives in normal wood and compression wood (%).

Solvent	*Abies sachalinensis* (*112*)		*Larix leptolesis* (*90*)	
	NW	CW	NW	CW
Ethyl ether	2.99	4.01	1.19	0.92
Ethanol-benzene (1:2)	0.98	0.98	2.54	3.82
95% Ethanol	0.52	0.59	0.29	1.10
Acetone-water (9:1)	0.14	0.45	-	-
Total extractives	4.63	6.03	4.02	5.84

2.9 Inorganics in compression wood

A range of elements including Ca, K, Mg, Cr, Al and Mn are incorporated into the plant cell wall (*161*). It has been reported that CW has higher levels of such elements than normal wood (*162,163*) (Table 2.16). The high concentration of Ca in CW may

be linked to its role in the lignification pathway since Ca is associated with highly lignified cell walls (*163*).

Table 2.16 Element concentrations in Norway spruce CW and opposite wood (ppm) (*162*).

	Al	Cr	Ca
CW	1.5	0.3	250
OW	0.5	0.05	50

2.10 References

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CHAPTER 3

3 Chemical changes associated with compression wood severity and juvenile wood, mature wood, earlywood and latewood formation in *Pinus radiata* compression wood

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3.1 Introduction

When a tree is displaced from its vertical position by external influences such as wind, snow or slope, compression wood is formed to restore its natural position. Compression wood forms in all conifer species and is very common in *Pinus radiata* (*1-3*). Log piles from almost any *P. radiata* forest in New Zealand are likely to contain batches of logs in which compression wood occupies up to 20% of the total log volume (*4*). This is a significant proportion of the merchantable volume of forest trees. Compression wood is generally considered to be inferior for both pulp and solid wood products (*5*,*6*).

Compression wood is anatomically, chemically and physically different from normal wood (*7*). Compression wood tracheids are shorter in length and have a more rounded shape with intercellular spaces, and cell walls have a highly lignified outer secondary ($S2_L$) layer, but no tertiary (S3) layer (*8*).

The most obvious chemical differences in chemical composition and lignin structure between compression wood and normal wood are that compression wood has a higher lignin and galactose content, a lower cellulose content and a higher percentage of *p*-hydroxyphenyl (H) units in its lignin (*7*). Compression wood lignin is generally considered to be more condensed than the lignin of normal wood (*7*).

Degrees of compression wood development may be encountered, and these form a continuum between normal wood, mild compression wood, and severe compression wood (*1*,*9-12*). Severe compression wood can be identified by its darker appearance in wet wood samples (*13*). Mild compression wood is a very common feature of plantation-grown radiata pine. It is often undistinguishable from normal wood unless

examined under the microscope. Microscopic examination is the best method to use for classifying compression wood severity but it is only applicable to small wood samples.

The overall goal of this investigation was the development of a quantitative chemical method for detection and/or grading of compression wood. Such a test would provide a valuable complement to microscopic methods, as it would have potential for determining the average compression wood content of a larger sample volume than is possible with microscopy.

In order to assess the potential of a chemical test for compression wood it is essential to understand the chemical changes that occur as the compression wood severity changes. In all *P. radiata* wood the chemical composition of the cell wall varies with both age and position within the tree (*14,15*). For example in a stem, there is a decrease in lignin content and an increase in cellulose content from pith to bark. A knowledge of the effect of this radial variation is essential for the development of a chemical test for compression wood. So far as is known, there has been no previous investigation of the effect of severity and wood age on the chemical composition of compression wood of *P. radiata*.

Differences in the chemistry of earlywood and latewood bands within an individual growth ring of *P. radiata* (*16*) have been reported. It is not clear whether differences in earlywood and latewood occur when compression wood is formed.

In the present study, changes in the chemical composition and lignin structure of compression wood were tracked across a single disc of *P. radiata.* In particular, variations between normal, mild and severe compression wood; between juvenile wood and mature wood; and between earlywood and latewood within an individual growth ring were recorded.

3.2 Materials and methods

A 5 cm thick stem disc was collected 5 m above the base of a *P. radiata* tree just above the point at which the terminal leader had been lost and an adjacent branch had

grown to become the new leader. The disc had 20 growth rings. Severe compression wood, mild compression wood and opposite wood were separated from growth rings 3-4, 10 and 15-16 (Figure 3.1) using a small band saw. Rings 3-4 represented the juvenile wood while rings 15-16 represented mature wood. Two growth rings were required from rings 3-4 and 15-16 to obtain sufficient material for analysis in the opposite wood area. Earlywood and latewood bands were separated from ring 10 using a sharp knife.

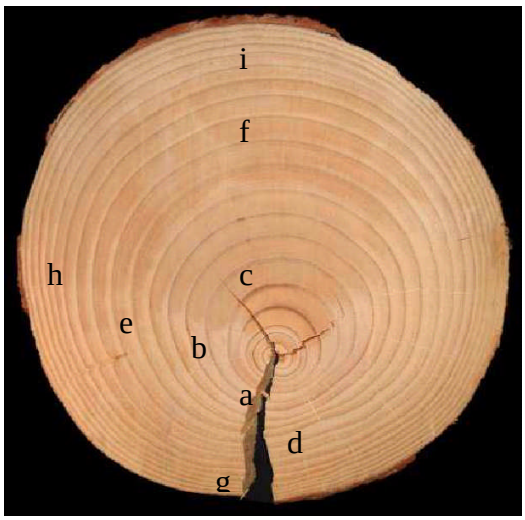


Figure 3.1 *P. radiata* disc growth rings and sampling positions. a = Rings 3-4 opposite wood, b = Rings 3-4 mild compression wood, c = Rings 3-4 severe compression wood, d = Ring 10 opposite wood, e = Ring 10 mild compression wood, f = Ring 10 severe compression wood, g = Rings 15-16 opposite wood, h = Rings 15-16 mild compression wood, i = Rings 15-16 severe compression wood.

Use of a single disc avoided within- and between-tree variation. Mild compression wood was present in the transition zone from normal wood to severe compression wood in this disc. Each compression wood sample was matched with a sample of wood from the same growth ring on the opposite side of the stem. This allowed separation of the effects of compression wood development from those associated with wood age. It was assumed that within a growth ring the circumferential variation was due solely to the presence of compression wood.

The degree of compression wood development was determined by fluorescence microscopy (*12*). It was only possible to describe three categories by this method:

normal; mild; and severe. Finer distinctions were not possible because the anatomical and uniform appearance could not be identified by microscopy.

All wood samples were air-dried, ground in a Wiley mill to pass a 20-mesh screen, extracted with dichloromethane overnight in a Soxhlet extractor and reground to pass a 60-mesh screen. Klason and acid-soluble lignins were determined in duplicates according to Tappi Standards T222 om-88 and UM 250 respectively. Monomeric carbohydrates in the filtrates from Klason lignin determination were determined by ion chromatography (*17*) and results expressed as anhydro sugar units. Anion exchange chromatography was performed on a Dionex CarboPac PA-1 anion column (Dionex Corp., Sunnyvale, California) (250 × 4 mm i.d.) using a model 4000i Dionex pump and controller system, a Dionex model UEM-1 pulsed amperometric detector, and a Waters model 710 WISP autosampler injector. The analytical column was protected by an AG6 guard column (50 × 4 mm i.d.). Freshly filtered-deionized water degassed with helium for 15 min prior to use was used as the eluent at a flow rate of 1 mL/min. 25 μL of wood hydrolysate was filtered (Titan syringe filters, nylon pore size 0.45 μm) and loaded onto the column. A standard solution containing the five sugars in the approximate proportions found in wood, plus the D-fucose internal standard, was used to determine response factors.

Length-weighted contoured fibre lengths and coarseness were determined using Metso FibreLab V3.0 analyser as described by Kibblewhite *et al*. (*18*). Pith to bark strips of tangential width ca 15 mm were cut from a 5 mm thick stem disc, notches were sawn 2 mm deep into the strips on the earlywood side of the latewood band, to mark growth layer positions. Fibre separation was obtained by kraft pulping the strips supported, between perforated stainless steel plates alone their radial faces as described in Kibblewhite *et al*. (*18*). The plates allowed liquor penetration while preventing damage to softened wood. Eight sets of plates/strips were placed in the digester, with the pith-end uppermost, for each pulping run. Radiata pine chips were added to the digester to the wood charge to 500 g o.d., and to ensure uniform liquor cover and circulation. Pulping conditions: effective alkali 16%, sulphidity 30%, liquor to wood ratio 8:1, overnight soak of chips in liquor at room temperature, time to 170 °C 3 hours, time at 170 °C 3 hours. The pulped strips were held intact between the perforated plates when washed under running water. Plates were removed from the washed strips and each growth layer split off along the grain at the

notches previously cut at their latewood/earlywood boundaries. Disintegration of the individual growth-layer samples was done by stirring in a baffled disintegration vessel (diameter 90 mm, height 200 mm, three vertical 5 × 5 mm square baffles, and a non-cutting, three paddle propeller stirrer. The never-dried, individual growth layer samples were diluted to 5 L, stirred, and subsampled for fibre length measurements.

Density and microfibril angle were determined by the CSIRO Wood Quality Laboratory, Melbourne, by SilviScan analysis (*19,20*). Acetyl content was determined using the signal area between 20 and 23 ppm relative to the total area of the spectrum acquired using a standard ^{13}C CPMAS NMR pulse sequence as described by Newman *et al*. (*21*).

3.2.1 Fluorescence microscopy

Transverse sections, 60 μm in thickness mounted in 70% glycerol, were examined with a Leica TCS NT confocal laser scanning microscope (Model TCS NT; Leica, Wetzlar, Germany) using the 488 nm line of an Ar-Kr laser for excitation and light at 515 nm for imaging (*12*). Lignification in the cell corner middle lamella and secondary wall was identified using lignin autofluorescence.

3.2.2 Releasable β-O-4 units

Duplicate determinations of the thioacidolysis monomers were carried out on 10 mg of pre-swollen wood (*22*) using the method of Pasco and Suckling (*23*). Levels of trithioethers were monitored by gas chromatography-mass spectrometry (GC-MS); analyses were performed on an HP 5890, Series II gas chromatograph interfaced with an HP 5971A Mass Selective Detector (MSD). The column was an HP-Ultra-2, 50 m x 0.2 mm i.d. with 0.25 μm film thickness. Helium was used as the carrier gas at a velocity of 25 cm/sec and a column head pressure of 20 psi. Injections (1 μL) were performed using a purged splitless technique (HP 7673A auto sampler) with a split flow of 20 mL/min after injection and the injector purged after 1 minute. The GC conditions were as follows: injector temperature, 250ºC; temperature program 40ºC (1 min) + 10ºC/min to 120º C + 4ºC/min to 240ºC (15 min) + 10ºC/min to 300ºC (10 min).

3.2.3 Size exclusion chromatography

Molecular weight profiles of the thioacidolysis products were performed at ambient temperature using three 300 × 7.8 mm Ultrastyragel columns (Waters Associates, linear, 500 Å and 100 Å) connected in series with HPLC grade tetrahydrofuran (0.6 mL/min) as the eluent and UV detection (Waters 2487 UV detector) at 280 nm. Thioacidolysis products were dissolved in tetrahydrofuran (ca. 4 mg/ mL), filtered and 20 μL injections were used (*24*).

3.2.4 Thioacidolysis/^{31}P NMR spectroscopy

Following the method of Smit *et al.* (*25*), thioacidolysis of the pre-swelled duplicate wood samples (100 mg) was carried out using dioxane (27 mL), ethanethiol (3 mL) and BF_3 etherate (0.75 mL) in PTFE-lined stainless steel reactors (110°C, 4 h). The cooled reaction mixture was transferred to a separating funnel and a known amount of internal standard (cholesterol, *ca*. 10 mg) added. The pH of the aqueous of was adjusted to 5, extracted with dichloromethane and the resulting extract dried and concentrated under reduced pressure.

A portion of the dry thioacidolysis product (*ca.* 30 mg) was dissolved in pyridine/chloroform-*d* (0.5 mL, 1.6/1 v/v) and 100 μL of the relaxation reagent (chromium(III) acetylacetonate, 5.0 mg/mL in the same solvent) followed by 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (**1**) (100 μL) added. The solution was made up to 1 mL and analysed by quantitative ^{31}P NMR spectroscopy. Spectra were collected using a 5 mm probe on a Bruker Avance NMR spectrometer, operating at 161 MHz. Inverse gated decoupled quantitative spectra were collected using 90° pulse, a sweep width of 25000 Hz and a delay of 20 s. At least 750 scans were collected for each sample. Chemical shifts were referenced to the reaction product of **1** with water, which has been observed to give a signal in pyridine/chloroform-*d* at 132.2 ppm.

3.3 Results and discussion

3.3.1 Anatomical characterisation of wood samples

Cross-sectional features of Rings 3-4 opposite wood, mild compression wood and severe compression wood are shown in Figure 3.2. The tracheids of the opposite wood tended to be rectangular in shape (Figure 3.2 (i)). There were no intracellular spaces between adjacent cells and lignification was most pronounced in the cell corner middle lamella (indicated by the brightest areas, arrow).

Mild compression wood tracheids resembled those of normal wood in their six-sided cross sectional shape with slightly rounded appearance. Mild compression wood was identified by the occurrence of a high degree of lignification in the $S2_L$ layer near the cell corners (arrow in Figure 3.2 (ii)). The absence of an S3 layer, the presence of intercellular spaces and the rounded shape of the cells are not consistent features of the mildest forms of compression wood (*11*).

In severe compression wood (Figure 3.2 (iii)), the tracheids had a rounded cell outline. Intercellular spaces were present at the corners. The cell walls of the tracheids, were thicker than those of the tracheids in opposite wood. Bright areas around the perimeter of the cell wall in severe compression wood indicated that the $S2_L$ was highly lignified around the periphery of the cell wall (arrow). Because of the high frequency of intercellular spaces, relatively little lignin was located in the cell corner compound middle lamella. A highly lignified $S2_L$ layer is a characteristic of compression wood, occurring in both mild and severe forms. It is common to compression wood in Ginkgoales and Taxales as well as the Coniferales (*11*). The absence of an S3 layer and the presence of helical checks are features that are not demonstrated in Figure 3.2 (iii).

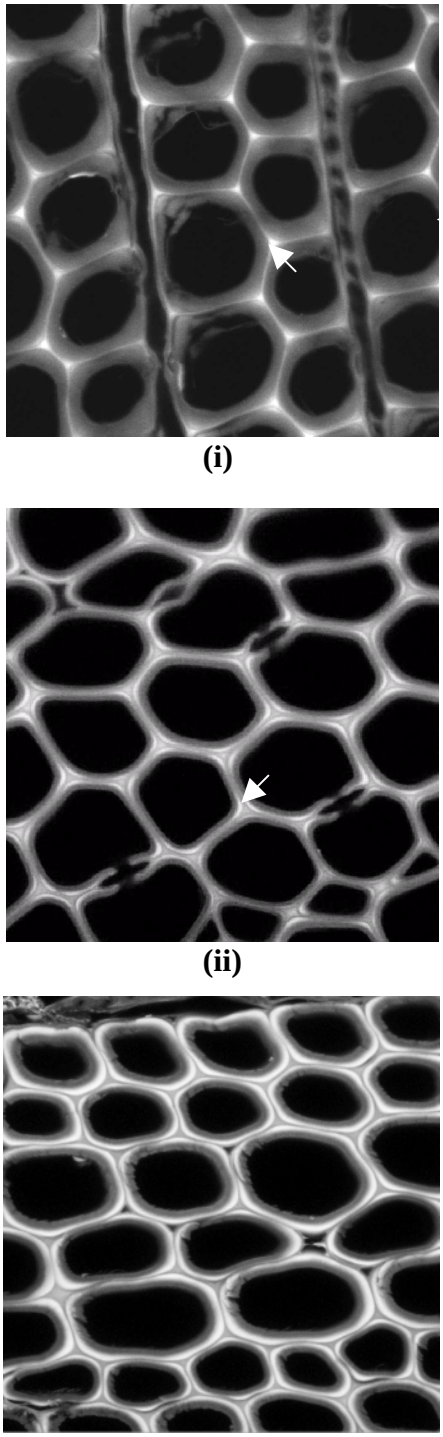


Figure 3.2 Confocal fluorescence images of cross-sections of *P. radiata* wood showing lignin autofluorescence. Opposite wood (i); mild compression wood (ii); severe compression wood (iii). Field of view 159 x 159 μm.

3.3.2 Morphology and physical properties of fibres

Data for fibre quality and physical properties are shown in Table 3.1. Fibre lengths of samples decreased gradually in the order opposite > mild compression wood > severe compression wood. Mature wood (Rings 15-16) fibres were longer than juvenile wood (Rings 3-4) fibres. These results agree with those of other studies of *P. radiata* compression wood (*18*,*26*). Coarseness of the compression wood was greater than that of opposite wood throughout, probably due to the development of thicker cell walls and shorter fibres. Trends in wood density were not very clear. Severe compression wood had a greater density than opposite wood, but mild compression wood did not. Juvenile wood had lower density than mature wood in normal wood and mild compression wood but not in severe compression wood. Microfibril angle (MFA) increased with increasing severity of compression wood development. This was a very clear trend. Results showed a decrease in MFA from pith to bark in both degrees of severity studied. The density and MFA values showed that the wood samples exhibited typical anatomical properties as reported in the literature for *P. radiata* (*12*).

Table 3.1 Fibre morphology and physical properties.

Sample	Fibre length (mm)	Coarseness (mg m^{-1})	Density (kg m^{-3})	MFA (°)
Rings 3-4				
OW	2.39	0.14	482	14.9
MCW	2.29	0.16	450	21.1
SCW	2.15	0.22	571	35.8
Ring 10				
OW			477	11.2
MCW			416	16.0
SCW			606	28.7
Rings 15-16				
OW	3.89	0.18	518	9.8
MCW	3.84	0.20	468	17.6
SCW	3.43	0.21	547	20.9
LSD[1] (*27*)	0.13	0.015	-	-

[1] LSD = least significance difference for p = 95%.

3.3.3 Basic chemical composition

The chemical composition of opposite wood, mild compression wood and severe compression wood differed in terms of lignin and monomeric sugar contents (Table 3.2).

Table 3.2 Basic chemical composition of opposite wood, mild compression wood and severe compression wood of stem disc Rings 3-4, 10 and 15-16.

Sample	Composition (g/100 g oven dry extractive-free wood)						Acetyl Content
	Lignin[1]	Arabinose[2]	Galactose[2]	Glucose[2]	Xylose[2]	Mannose[2]	%
Rings 3-4							
OW	27.9	1.9	4.5	41.1	6.7	9.0	1.2
MCW	32.9	1.6	8.0	35.0	5.9	7.2	0.9
SCW	38.6	1.4	14.0	28.2	5.0	5.0	0.8
Ring 10							
OW	27.3	1.7	2.4	45.1	6.5	10.6	1.2
MCW	31.4	1.6	4.7	43.3	5.3	10.9	1.0
SCW	35.4	1.3	10.1	35.0	5.1	6.7	0.7
Rings 15-16							
OW	27.1	1.3	1.8	46.6	4.6	11.7	1.3
MCW	31.3	1.2	4.5	39.7	4.6	9.0	0.9
SCW	35.6	0.9	8.2	33.8	4.6	6.9	0.8
LSD[3]	1.1	0.2	0.3	3.1	0.9	1.3	-

[1] Klason plus acid soluble lignin.
[2] Expressed as anhydro sugar units.
[3] LSD = least significance difference for p = 95% (Appendix A).
OW = opposite wood, MCW = mild compression wood, SCW = severe compression wood.

For comparison of means within the columns Least Significant Difference (LSD) values were calculated. If the difference between the two means are larger than the LSD value then those values are considered to be significantly different. Least Significant Difference for duplicate analyses was calculated by repeated analyses of eighteen *P. radiata* control wood samples over a period of 3 weeks (Appendix A).

The lignin content of mild compression wood was 18% higher while severe compression wood lignin content was 30-38% higher than that of opposite wood.

There was a good agreement between the anatomical classification of the wood samples and their lignin content (Chapter 4, Table 4.2).

Amounts of monomeric sugars also appeared to be related to the degree of development of compression wood. Galactose levels increased with compression wood severity while glucose and mannose levels decreased. Such changes are associated with elevated levels of galactan (*28,29*) and lower proportions of cellulose and galactoglucomannan (*30*) present in compression wood. Xylose and acetyl content decreased with compression wood severity except in mature wood, where the xylose content remained constant. In the literature, conflicting results have been reported for the xylose content of compression wood and opposite wood. In *P. sylvestris* and *P. resinosa* a considerable decrease in xylose content has been reported in compression wood (*30*). In the present study the amounts of arabinose residues were lower in compression wood than in opposite wood.

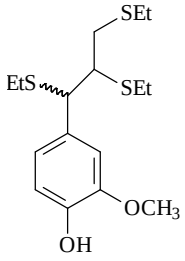
Chemical differences between opposite wood and severe compression wood were very similar to those reported previously (*30*). Data for the mild compression wood are reported here for the first time. Results show that the summative composition of mild compression wood was intermediate between that of normal wood and severe compression wood. This was observed in all ages of wood examined.

Radial variation of chemical composition within the opposite wood samples was apparent especially between Rings 3-4 and Rings 15-16. Ring 10 was considered to be the transition zone between juvenile and mature wood. In comparison with Rings 3-4, Rings 15-16 had a slightly lower levels of lignin, arabinose, galactose and xylose, while glucose and mannose content was slightly higher. These trends agree with data reported for *P. radiata* (*31,32*) and other species (*33,34*). Trends in chemistry similar to those in opposite wood were observed in compression wood from Rings 3-4 and 15-16, which is in agreement with literature (*34*). The chemistry of compression wood is influenced by both age and severity of compression wood formation. The observed variation in chemical composition between juvenile compression wood and mature compression wood was partly due to wood age but also due to differences in compression wood severity between Rings 3-4 and 15-16. Anatomically the compression wood in Rings 3-4 was more severe than compression wood from Rings 15-16 (Table 4.2). It was therefore impossible to separate one

effect from the other in this study. Larson (*33*) examined the effect of age on compression wood formed within the first nine growth rings in a young *P. resinosa* tree. No firm conclusions could be drawn since anatomical information regarding the severity of samples examined was lacking.

3.3.4 Lignin structure

In this study thioacidolysis was used to determine the levels of releasable guaiacyl (G) and *p*-hydroxyphenyl (H) β-O-4 linkages of the lignin (*35*) which were measured as the trithioethers (**2**) and (**3**) respectively. Only a portion of total β-O-4 ether units i.e. those that are either phenolic or linked at C4 via an ether bond to the α or β position of an adjacent unit in the lignin, can be measured in this way. For example, β-O-4 units, linked to other units by a C-C bond at C-5 are not converted to the monomers **2** or **3** on thioacidolysis. Some authors refer to units that are amenable to analysis in this way, as "uncondensed β-O-4 units". In order to avoid confusion the term "releasable β-O-4 units" is used here.



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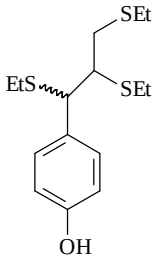


3

The results of thioacidolysis are shown in Figure 3.3. The levels of releasable H β-O-4 units increased and those of releasable G β-O-4 units decreased with compression wood severity. It is clear that compression wood lignin differed from opposite wood lignin in its lower content of releasable G β-ethers and higher levels of releasable H β-ethers. In severe compression wood and mild compression wood releasable H units comprised ~17% and ~6% respectively of the total yield respectively while in opposite wood the releasable H units accounted for only 1%. This observation is in agreement with the literature data reported for *P. radiata* (*36*) and other species (*34,37*). The total thioacidolysis yield (**2**+**3**) from compression wood and opposite wood was not significantly different. This observation concurs with published work (*35,38*) but contradicts the generally accepted concept that compression wood lignin is more condensed than normal wood, and gives a lower yield from thioacidolysis.

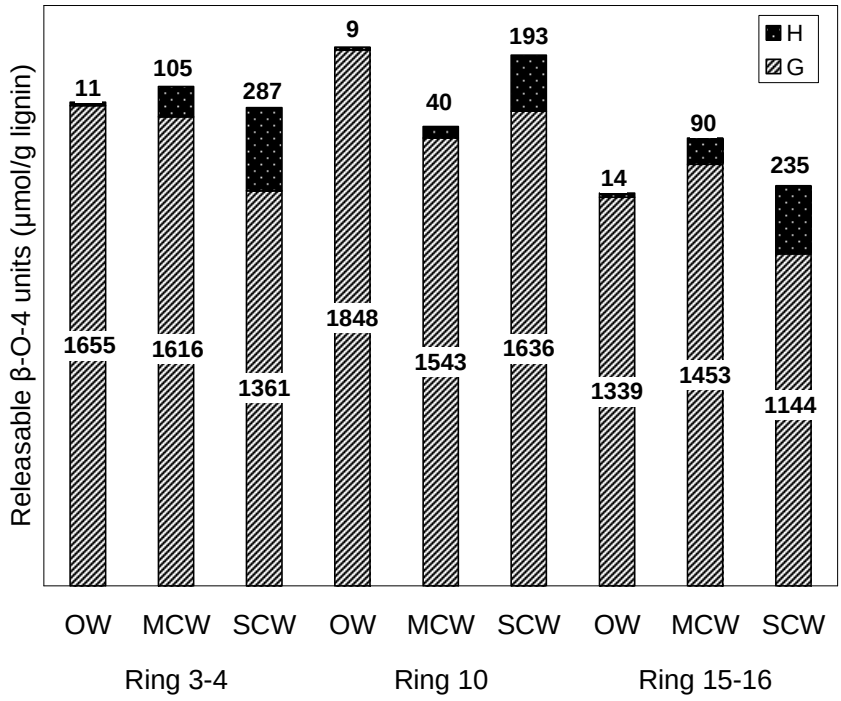


Figure 3.3 Yield of releasable β-ethers. OW = opposite wood, MCW = mild compression wood, SCW = severe compression wood, H = *p*-hydroxyphenyl, G = guaiacyl.

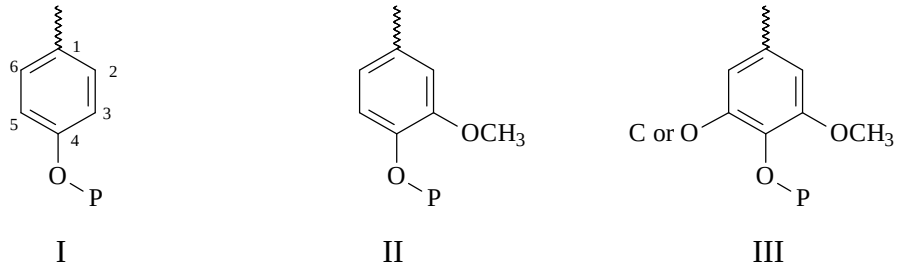
Within the single stem disc examined, the total thioacidolysis yield was lower for Rings 15-16 compared to Rings 3-4 or Ring 10, for both opposite wood and compression wood. Yeh *et al*. (*34*) reported higher nitrobenzene oxidation yield in juvenile wood (Rings 5-8) than in mature wood (Rings 19-27) of *P. taeda* L. This observation was true for both opposite wood and compression wood samples, and is in agreement with our results.

Releasable β-ether yields were 25-40% higher than those previously reported for *P. radiata* normal wood (*23*). This was due to the pre-swelling of wood samples in water prior to thioacidolysis (*22*).

3.3.5 Thioacidolysis/^{31}P NMR spectroscopy

Thioacidolysis/^{31}P NMR spectroscopy has been used to determine uncondensed and condensed C9 units in *in situ* lignin (*25,39*) (Figure 3.4). Most lignin characterisation techniques account for only 30-50% of lignin and, may not give a true representation

of the nature of lignin in the cell walls. Thioacidolysis/^{31}P NMR spectroscopy is the first technique to account for approximately 75% of total C9 units in lignin. It gives a much better representation of the cell wall lignin (*25*).



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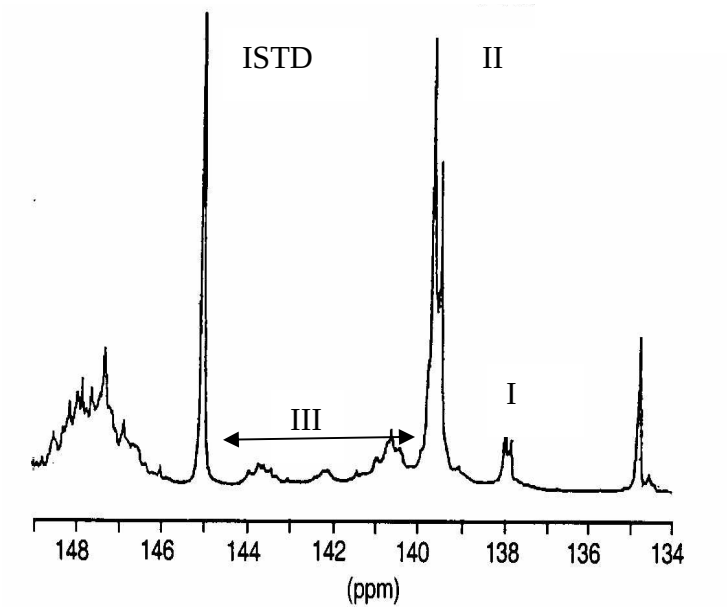

Figure 3.4 ^{31}P NMR spectra of phosphitylated thioacidolysis product from compression wood showing chemical shifts related to an internal standard (ISTD), uncondensed H (I), uncondensed G (II), and condensed G (III) C9 units.

Results of ^{31}P NMR analysis showed that levels of uncondensed H C9 units rose with increasing compression wood severity, reaching 14% of the total measured C9 units (Table 3.3). Mild compression wood samples had an uncondensed C9 H unit content intermediate between those observed for opposite wood and severe compression wood. Assuming an average molecular weight of 183 g/mol for a lignin C9 unit this method accounted for approximately 77% of the total C9 units in lignin. Table 3.3 shows that the condensed guaiacyl C9 units, i.e. those substituted at C5 by O or C, increased only slightly with compression wood severity, while uncondensed guaiacyl C9 units decreased. As a result the proportion of condensed G C9 units increased with severity (33-39%). This observation is consistent with the thioacidolysis results,

in which the levels of releasable G β-O-4 units decreased with increasing compression wood severity.

Mature wood had higher levels of condensed 5-5 C9 units than juvenile wood, but no difference was observed in percentage of condensed G C9 units (39%) between juvenile wood and mature wood even though the total thioacidolysis yield was lower in the Rings 15-16 indicating a more condensed lignin.

Table 3.3 Thioacidolysis/^{31}P NMR spectroscopy results.

Sample	C9 units (mmol/g lignin)							%
	β-5	4-O-5	5-5	Cond. G[1]	Uncond. G	Uncond. H	Total	Cond. G
Rings 3-4								
OW	0.32	0.28	0.66	1.26	2.51	0.08	3.86	33
MCW	0.38	0.20	0.74	1.32	2.33	0.26	3.89	36
SCW	0.38	0.29	0.67	1.34	2.08	0.56	3.99	39
Ring 10								
OW	0.40	0.25	0.67	1.32	2.66	0.07	4.05	33
MCW	0.45	0.25	0.61	1.31	2.51	0.11	3.93	34
SCW	0.42	0.26	0.70	1.38	2.40	0.41	4.19	37
Rings 15-16								
OW	0.36	0.25	0.71	1.31	2.50	0.05	3.87	34
MCW	0.38	0.20	0.75	1.32	2.31	0.19	3.82	36
SCW	0.34	0.23	0.84	1.40	2.20	0.37	3.97	39
LSD[2]	0.08	0.07	0.12	0.18	0.16	0.02	-	-

[1] Condensed G = sum of 5-5, 4-O-5 and β-5 C9 units.
[2] LSD, least significance difference for $p = 0.05$ (Appendix A).
OW = opposite wood, MCW = mild compression wood, SCW = severe compression wood.

Least significant difference (LSD) for duplicate analyses was calculated by repeated analyses of six *P. radiata* control wood samples over a period of 2 weeks.

Thioacidolysis/^{31}P NMR data showed no significant difference in the levels of guaiacyl C5 condensed C9 units in compression wood and normal wood. This contradicts much of the earlier literature (*7,40,41*), in which compression wood lignin was held to be a more condensed polymer formed by bulk polymerisation. On

the other hand the results of the present study agree well with the current view that compression wood lignin is formed by end-wise polymerisation and is therefore not significantly different from normal wood lignin (*25,42*).

3.3.6 Degree of lignin condensation

The amounts of oligomers present after thioacidolysis (particularly the higher molecular weight fraction) increases slightly with increasing severity of compression wood formation in Rings 3-4 (Figure 3.5). This indicated that as compression wood develops, the proportion of C9 units linked by bonds which are cleaved by thioacidolysis decreases; i.e. the lignin becomes more condensed. Mild compression wood lignin had a degree of condensation intermediate between that of opposite wood and severe compression wood. The trend was similar for Ring 10 and Rings 15-16. The SEC profiles of thioacidolysis products resemble those for thioacidolysed compression wood of *P. radiata* and *Picea abies* (*22,24*).

The peak corresponding to the oligomers was only slightly different (data not shown) for juvenile and mature compression wood. This suggests that the levels of condensed structures in juvenile wood and mature wood were similar.

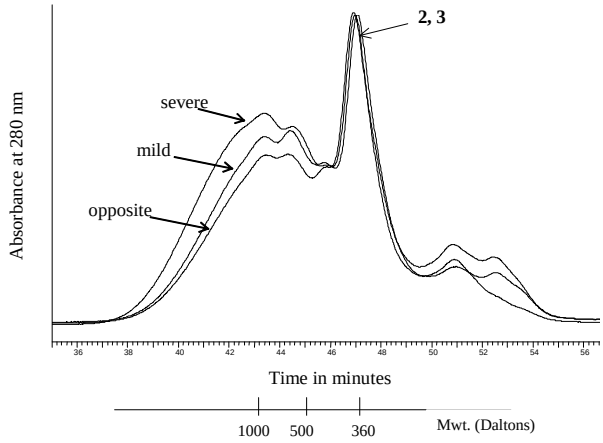

Figure 3.5 Size exclusion chromatogram of the thioacidolysis products from juvenile wood. Peaks eluting after the monomers (2,3) are not lignin-related (*22*). Chromatograms were normalized with respect to the most intense (monomer) peak.

In contrast to the findings from thioacidolysis/^{31}P NMR spectroscopy the thioacidolysis/SEC results and previous reports on SEC (*22,24*) indicate a slightly higher proportion of condensed structures (structures condensed at C2, C5, C6 and at

β) in compression wood than in normal wood. This suggests that in compression wood, C2, C6, β-β and β-1 condensed structures may be present at higher levels than in normal wood. No evidence to support this argument could be found in the literature. It should be emphasised that thioacidolysis/SEC is a qualitative technique and that exact comparisons are not possible.

3.3.7 Comparison between compression wood and juvenile wood

Juvenile wood or core wood formed near the stem centre has been shown to contain higher levels of lignin, xylan and glucomannan and lower level of cellulose than mature wood (*43*). It is thought to resemble compression wood in these respects (*43*). Results of this study clearly showed that the chemical composition and lignin structure of juvenile wood (Rings 3-4) was different from that of compression wood, in contrast to some literature reports (*43*). Compression wood lignin could be distinguished from juvenile wood lignin by its significantly greater amount of releasable H β-ether units. Yeh *et al*. (*44*) compared juvenile wood from 9 month old *Pinus taeda* plants with the compression wood formed after artificial bending of plants grown under similar conditions. Analysis of the two types of wood showed that juvenile wood was chemically different from compression wood.

3.3.8 Earlywood (EW) and latewood (LW) in compression wood

Basic chemical composition of EW/LW from Ring 10 is shown in Table 3.4. The differences in lignin content and monomeric sugars between EW and LW in opposite wood are in good agreement with data reported for *P. radiata* (*16,45*) and other species (*33,46,47*).

The results indicated that the lignin content of EW and LW in mild and in severe compression wood was not significantly different. This is consistent with the anatomical features (not shown). In compression wood there was very little difference in the thickness of EW and LW cell walls, whereas in opposite wood the LW tracheid walls were thicker than those in EW. Therefore, the proportion of lignin contributed by the highly lignified middle lamella was correspondingly higher in the thin walled EW for opposite wood, leading to greater differences in the lignin content between EW and LW. According to Timell (*7*) the “earlywood” and “latewood” terms are not applicable to compression wood. It is the first-formed

tracheids that are different from the other tracheids present in the growth ring. The chemical composition of the two tissue types has been reported to be very similar (*46*).

Table 3.4 Chemical composition of earlywood and latewood in Ring 10.

		Component (g/100 g oven dry extractive-free wood)						Releasable β-O-4 units (μmol/g lignin)		
Sample		Lignin	Ara	Gal	Glc	Xyl	Man	G	H	H/G
OW	EW	27.6	1.5	1.4	48.0	5.8	11.1	1,643	9	0.01
	LW	26.4	1.6	1.8	49.7	6.0	12.9	1,739	8	0.01
MCW	EW	31.9	1.4	4.5	43.6	6.0	9.4	1,491	33	0.02
	LW	30.8	1.6	4.4	42.0	5.7	11.0	1,645	19	0.01
SCW	EW	36.5	1.2	10.0	35.8	4.9	6.7	1,497	187	0.12
	LW	35.8	1.1	9.9	36.0	5.0	6.8	1,706	118	0.07
LSD[1]		1.1	0.2	0.3	3.1	0.9	1.3	237	6	-

[1] LSD, least significance difference for $p = 0.05$ (Appendix A).
OW = opposite wood, MCW = mild compression wood, SCW = severe compression wood, EW = earlywood, LW = latewood.

There was no significant difference in the amounts of releasable G β-ethers (Table 3.4) produced from EW and LW. The proportion of releasable H β-ether units was higher in EW than in LW. No other studies have been found with which to compare these results.

In the SEC curves (not shown) for thioacidolysis products of EW and LW in severe compression wood, the area corresponding to the oligomer peak for the EW was slightly higher than that for LW. This was consistent with thioacidolysis results.

According to the thioacidolysis/^{31}P NMR results (Table 3.5), EW had a greater proportion of uncondensed H C9 units, which is consistent with the thioacidolysis results. The condensed G C9 units were not different between EW and LW. During microscopic examination, it was found that EW and LW stained differently with some dyes. This may indicate that EW and LW differ in their lignin structure. On the other hand it has been reported that there is no difference between EW and LW in terms of softening properties (*48*). Differences would be expected due to the structural differences between the lignin in the middle lamella and the secondary

wall. Unfortunately, no definite conclusion can be reached from results obtained from only one growth ring, and further studies are necessary.

Table 3.5 Thioacidolysis/^{31}P NMR spectroscopy results.

Sample	C9 units (mmol/g lignin)							%
	β-5	4-O-5	5-5	Cond. G	Uncond. G	Uncond. H	Total	Cond. G
Opposite								
EW	0.42	0.30	0.68	1.40	2.55	0.02	3.97	35
LW	0.44	0.24	0.64	1.38	2.61	Trace	3.98	35
Mild								
EW	0.43	0.28	0.58	1.29	2.44	0.16	3.89	35
LW	0.40	0.19	0.67	1.26	2.50	0.09	3.85	34
Severe								
EW	0.34	0.25	0.77	1.36	2.26	0.35	3.97	38
LW	0.39	0.22	0.66	1.26	2.26	0.23	3.94	34
LSD[1]	0.08	0.07	0.12	0.18	0.16	0.02	-	-

[1] LSD, least significance difference for $p = 0.05$ (Appendix A).

3.4 Conclusions

Study of compression wood within a single disc of *Pinus radiata* showed that increasing severity of compression wood is associated with increases in lignin and galactose levels and decreases in glucose and mannose levels. Changes in the structure of lignin were also associated with increasing severity. Levels of releasable *p*-hydroxyphenyl β-ethers increased, while those of releasable guaiacyl β-ethers decreased. Levels of uncondensed *p*-hydroxyphenyl C9 units increased, while uncondensed guaiacyl C9 units decreased. Proportions of condensed guaiacyl C9 units present in compression wood were similar to those in normal wood.

Differences in the chemical structure and composition of juvenile and mature compression wood were less marked. The results clearly showed that compression wood is different from juvenile wood in terms of chemical composition and lignin structure.

Earlywood and latewood components in compression wood had a similar basic chemical composition. Lignin in EW had higher levels of releasable H β-ethers and a higher proportion of uncondensed *p*-hydroxyphenyl C9 units. Due to the limited number of samples examined, it was impossible to draw definite conclusions about lignin structural differences in the lignin of EW and LW.

3.5 References

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CHAPTER 4

4 A quantitative indicator of the degree of compression wood development

4.1 Introduction

Pinus radiata is the most important plantation-grown commercial species in New Zealand. Like most fast-growing conifers, it is prone to develop compression wood as a response to any disturbance, such as stem displacement or loss of a terminal leader (*1*). The formation of compression wood allows the tree to regain its normal growth orientation. Log piles from almost any *P. radiata* forest in New Zealand will contain batches of logs in which compression wood accounts for up to 20% of the total log volume (*2*). Compression wood is vital to the living tree, but undesirable from the standpoint of timber utilisation. On drying it shrinks more than normal wood in the longitudinal direction, and this causes distortions in the sawn timber. Compression wood is harder and more brittle than normal wood. Kraft pulps prepared from compression wood give lower yields, than those from normal wood and require more bleaching.

All gradations of compression wood may be encountered in a continuum from normal to severe compression wood in plantation-grown *P. radiata* (*3-5*). Mild compression wood is common in fast growing conifer species such as *P. radiata*, but cannot be distinguished visually from normal wood and must be identified by microscopical examination (*6,7*). Severe compression wood is easily recognised from its dark colour and distinctive growth pattern.

Chemical differences between compression wood and normal wood have been observed (*8*). Compression wood contains more lignin and galactan and less cellulose and galactoglucomannan than normal wood. Compression wood lignin has a higher proportion of *p*-hydroxyphenyl units and a higher frequency of "condensed" structures than normal wood (*8,9*). To date chemistry of compression wood in *P. radiata* has been shown to be similar to that in other softwoods (*10-15*).

The overall goal of this investigation was the development of a quantitative chemical test for detection and/or grading of compression wood. The study was focused on understanding of chemical changes that occur with increasing compression wood severity, increasing wood age and the morphological origin of the samples.

In order to evaluate the usefulness of a chemical measure of severity, some sort of severity index is required. No available quantitative measure of compression wood severity has yet been devised, therefore a qualitative assessment was carried out using the degree of lignification of the cell wall as detected by fluorescence microscopy (*6,7*). It was not possible to chemically analyse the exact wood sample that had been characterised in this way because chemical analysis requires a much larger sample and microscopically only a very smaller area can be easily assessed.

Chapter 3 described chemical changes associated with compression wood severity and wood age within a stem disc of *P. radiata*. This Chapter assesses the basic chemical composition and lignin structure in a range of compression wood samples taken from branches, knots and young wood of *P. radiata*. Results were amalgamated with data from Chapter 3 to find out whether changes in chemistry can be used to measure compression wood severity, and if so, to identify the most useful chemical indicator.

4.2 Materials and methods

Compression wood and opposite wood samples, each 2 × 2 × 2 cm, were taken from branch and knots of a 10-year-old *P. radiata* tree and from stems of 1-year and 2-year old *P. radiata* plants. Pith and bark of young seedlings stems were carefully discarded.

4.2.1 Fluorescence microscopy

Transverse sections, 60 μm in thickness mounted in 70% glycerol, were examined with a Leica TCS NT confocal laser scanning microscope (Model TCS NT; Leica, Wetzlar, Germany) using the 488 nm line of an Ar-Kr laser for excitation and light at 515 nm for imaging according to the method described by Donaldson *et al*. (*6,7*). The severity of compression wood development in each sample was classified as

severe, mild or normal. Visible autofluorescence is primarily due to lignin and shows the distribution of lignin across the tracheid cell wall. Increased autofluorescence indicates increased lignification (*7*). An average of three examinations per sample was carried out. The following features were recorded:

1. Presence of a highly lignified $S2_L$ cell wall layer.
2. Presence of intercellular spaces.

4.2.2 Chemical analyses

All wood samples were air-dried, ground in a Wiley mill to pass a 20-mesh screen, then extracted with dichloromethane overnight in a Soxhlet extractor and reground to pass a 60-mesh screen. Klason and acid-soluble lignin were determined according to Tappi Standards T222 om-88 and UM 250 respectively. Monomeric sugars in the filtrates from Klason lignin determination were analysed by ion chromatography (*16*) and results were expressed as anhydro sugar units. Releasable β-O-4 units were determined as described in Chapter 3. Thioacidolysis/^{31}P NMR spectroscopy was performed as described in Chapter 3.

4.2.3 Pyrolysis

Extractive-free wood samples were packed into a fine quartz tube and pyrolysed at 600°C using an analytical pyrolysis unit (pyrojector II; SGE, Melbourne, Australia) attached to a gas chromatograph (GC8060; Fisons, Milano, Italy) interfaced with a mass spectrometer (VG Platform II; Fisons, Milano, Italy) controlled by a Masslynx^{TM} datasystem (Micromass Ltd.; Manchester, UK). Pyrolysis products were separated on a Supelcowax 10 column (30 m, 0.25 mm i.d., 0.25 μm film thickness). Helium was used as the carrier gas at a head pressure of 15 psi, with a purge flow of 20 mL/min. The injector port temperature was 250°C and the temperature program was 40°C (2 min) + 4°C/min to 250°C (20 min). Mass spectra were recorded in the electron impact mode (70 eV) with a mass range 40-400 amu. The pyrolysis products were identified by comparing retention times and mass spectra with those of reference samples and published data (*17-19*). The H/G ratio of lignin was calculated from the sum of all peak areas assigned to *p*-hydroxyphenyl (H) and guaiacyl (G) units.

4.3 Results and discussion

Compression wood samples contained significantly higher levels of lignin and galactose residues and lower levels of glucose, mannose, arabinose and mannose residues than opposite wood samples (Table 4.1). This is in agreement with results reported in Chapter 3 and also with published results of other studies cited in Chapter 3.

Chemical changes associated with compression wood formation in branch and young wood were similar to those observed in stem compression wood (Chapter 3). They were consistent with those previously reported for *P. radiata* (*11,12*) and other conifers (*8,20,21*).

Table 4.1 Chemical composition of *P. radiata* wood samples.

	Component (g/100 g oven dry extractive-free wood)						Releasable β-O-4 units (μmol/g lignin)		
Sample	Lignin[1]	Ara[2]	Gal[2]	Glc[2]	Xyl[2]	Man[2]	G	H	H/G
Branch									
OW	30.7	1.6	2.9	37.5	6.8	9.3	1,420	28	0.02
CW	39.6	1.1	12.2	25.4	4.2	5.8	1,057	393	0.37
Knot									
OW	30.1	1.7	2.7	35.7	6.3	9.4	1,526	40	0.03
CW	38.0	1.0	10.2	27.0	3.7	7.2	1,285	313	0.24
Stem (below and above branch)									
OW	29.9	1.6	2.4	40.4	5.8	11.0	1,305	18	0.01
CW	39.1	1.3	10.0	28.9	3.4	6.9	1,107	253	0.23
Young wood - 1 year									
OW	30.0	2.0	2.8	40.0	7.7	8.9	1,707	Trace[3]	-
CW	31.6	2.0	6.7	35.8	6.7	7.9	1,548	61	0.04
Young wood - 2 year									
OW	27.4	2.2	2.0	43.2	8.2	10.6	1,342	11	0.01
CW	34.9	1.6	9.0	33.7	5.3	8.2	1,401	127	0.09
LSD[4]	1.1	0.2	0.3	3.1	0.9	1.3	237	6	-

[1] Klason plus acid soluble lignin.
[2] Expressed as anhydro sugar units.
[3] Detection limit 9 μmol/g lignin.
[4] LSD, least significance difference for $p = 0.05$ (Appendix A).
OW = opposite wood, CW = compression wood.

The relationship between the measured lignin content and the microscopical classification was explored. Table 4.2 shows that there was a good agreement between compression wood severity assessed by fluorescence microscopy and the measured lignin content. Samples with higher lignin contents corresponded with more pronounced compression wood characteristics. Based on these results, lignin content above 31% was considered to indicate the presence of compression wood. Okuyama *et al*. (*22*) reported a positive correlation between lignin concentration in the secondary cell wall and growth stress which resulted in compression wood formation.

Table 4.2 Lignin content of *P. radiata* wood samples and degree of lignification assessed by fluorescence microscopy. The degree of lignification is indicated by the number of + signs.

Sample	Lignin content[1]	Lignification in cell corner middle lamella	Intercellular spaces	Lignification in $S2_L$ at cell corner	Lignification in $S2_L$ around cell wall
OW/MW	27.1	++			
OW/ring 10	27.3	++			
OW/2 year	27.4	++			
OW/JW	27.9	++			
OW/stem	29.9	++			
OW/1 year	30.0	++			
OW/knot	30.1	++			
OW/branch	30.7	++			
MCW/MW	31.3	+		+	
MCW/ring 10	31.4	+		+	
MCW/1 year	31.6	+		+	
MCW/JW	32.9	+		++	
MCW/2 year	34.9		+	+++	
SCW/ring 10	35.4		++	+++	++
SCW/MW	35.6		++	+++	++
SCW/knot	38.0		+++	+++	+++
SCW/JW	38.6		+++	+++	+++
SCW/stem	39.1		++++	+++	+++
SCW/branch	39.6		++++	+++	++++

OW = opposite wood, MW = mature wood, JW = juvenile wood, MCW = mild compression wood, SCW = severe compression wood, $S2_L$ = outer S2 layer; [1] g/100 g oven dry extractive-free wood.

A number of chemical parameters were plotted against lignin content in order to find the most reliable quantitative indicator of compression wood severity. Each indicator for measuring compression wood severity was assessed against the following criteria:

1. It must change linearly with the degree of compression wood severity.

2. It should span a wide range of levels relative to those normally found in wood, i.e. $\frac{CW_{\max} - OW_{mean}}{OW_{mean}}$ should be as large as possible.

3. It should be insensitive to the morphological origin of the sample, i.e. $\frac{CW_{\max} - OW_{mean}}{OW_{range}}$ should also be as large as possible.

While these were the key criteria for selection of parameters to measure, a number of other conditions had to be satisfied. The parameter had to be relatively easy to determine; determination should have a good level of accuracy; and the cost of determination should be relatively low.

4.3.1 Lignin content and levels of monomeric sugars as compression wood indicators

Figures 4.1 and 4.2 show relationships between lignin content and levels of monomer sugars in all wood samples. These indicate that, glucose, xylose and mannose changed approximately linearly with lignin content in all opposite wood and compression wood samples studied. However, galactose content stayed approximately constant in opposite wood (lignin content of 26-31%) but increased linearly ($R^2 = 0.79$)[1] when lignin levels exceeded ~31% i.e. as the compression wood severity increased. It was concluded that, even though compression wood formation was associated with changes in the levels of several monomer sugars, only the lignin and galactose contents are useful indicators of compression wood severity. Within-tree and between-tree variability account for much of the scatter in the data presented in Figures 4.1 and 4.2.

[1] R^2 values for this instance and all subsequent correlations are presented for compression wood samples only.

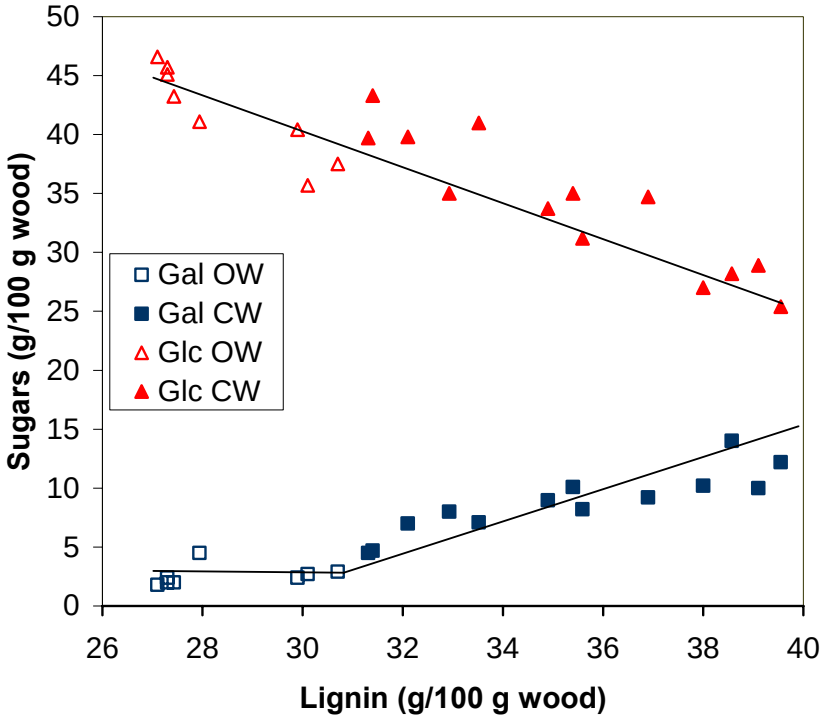


Figure 4.1 Relationships between lignin content and levels of galactose (Gal) (R^2 = 0.79) and glucose (Glc) in *P. radiata* wood samples.

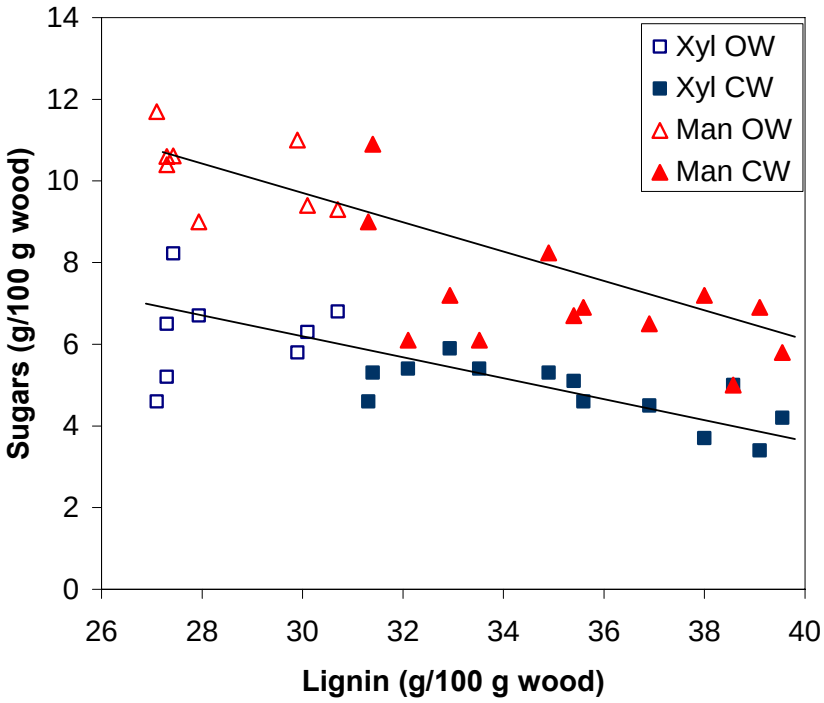


Figure 4.2 Relationships between lignin content and levels of xylose (Xyl) (R^2 = 0.48) and mannose (Man) (R^2 = 0.35) in *P. radiata* wood samples.

Newman *et al*. (*23*) reported close correlation between the galactose/glucose ratio and compression wood severity determined by visual assessment. The advantage of using a ratio of different sugars rather than a single sugar is that the need to detect absolute amounts is avoided. The relationship between galactose/glucose ratio and severity of compression wood formation in the present study is shown in Figure 4.3. Strong correlation (R^2 = 0.86) with lignin content shows agreement with the results of Newman *et al*. (*23*).

Yeh *et al*. (*24*) reported that the galactose/mannose ratio is a better indicator of the existence of compression wood than the galactose/glucose ratio. In the present study galactose/mannose ratio was weakly related to lignin content (R^2 = 0.63). The results are not shown.

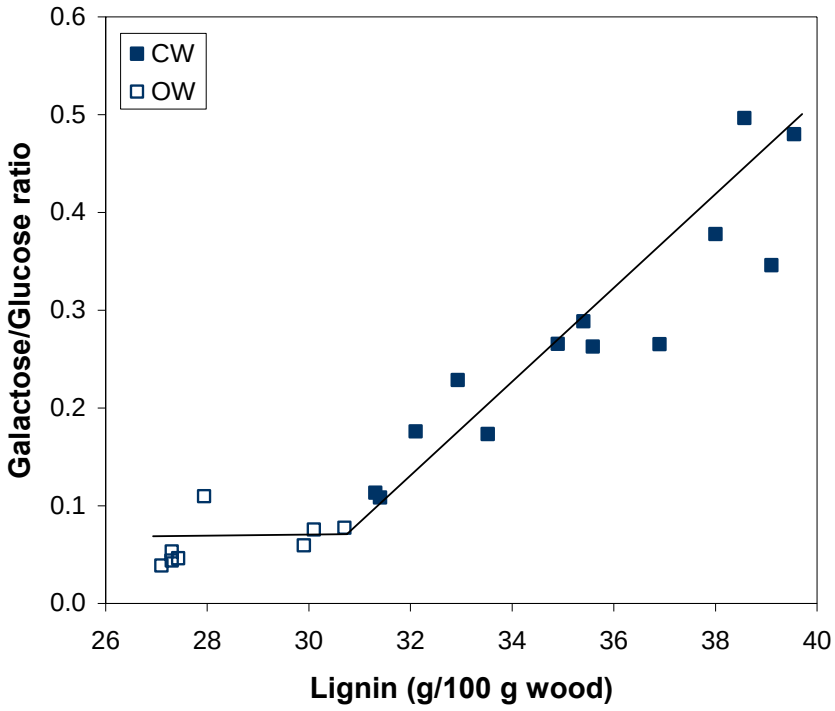


Figure 4.3 Relationship between lignin content and the galactose/glucose (R^2 = 0.86) ratio in *P. radiata* wood samples.

Galactose content and the galactose/glucose ratio were linearly related to compression wood severity i.e. both satisfied Criterion 1 (Figures 4.1 and 4.3). The galactose/glucose ratio spanned a larger range relative to levels found in normal wood (Criterion 2) and was less sensitive to the morphological origin of the sample (Criterion 3). The galactose/glucose ratio was therefore a better indicator of compression wood development than galactose alone (Table 4.3). Lignin content

correlated well with compression wood severity (Table 4.2), but the increase in lignin with compression wood severity was proportionately much smaller than that of galactose or the galactose/glucose ratio. This suggests that lignin content is less suitable as an indicator for compression wood severity than the monomeric sugar levels.

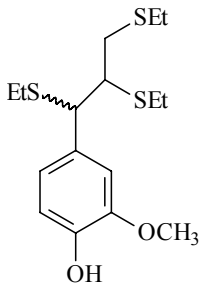
Table 4.3 Performance criteria for lignin content, galactose content and galactose/glucose ratio.

	(g/100 g wood)		Gal/Glc
	Lignin	Galactose	
OW_{mean}	28.5	2.6	0.06
OW_{max}	30.7	4.5	0.23
OW_{min}	27.1	1.8	0.04
CW_{max}	39.6	14.0	0.50
Correlation coefficient (R^2)	1.0[1]	0.79	0.86
$\frac{CW_{max} - OW_{mean}}{OW_{mean}}$	0.39	4	7.3
$\frac{CW_{max} - OW_{mean}}{OW_{range}}$	3.1	4.2	6.1

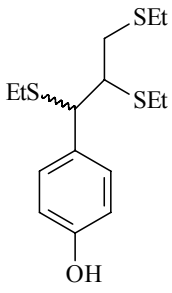
[1]By definition.

4.3.2 Lignin structural units as compression wood indicators

4.3.2.1 Releasable β-O-4 units



1



2

The levels of releasable G (**1**) and H (**2**) β-O-4 units were plotted against the lignin content of the wood samples (Figure 4.4). Although the levels of releasable H β-O-4 remained low and approximately constant in opposite wood, increase in compression

wood severity was associated with increase in the levels of releasable H β-O-4 units (R^2 = 0.81). A decrease in levels of releasable G β-O-4 units was observed as the severity of compression wood increased. The scatter among opposite wood data obscured any trend in the opposite wood data. Total releasable β-O-4 units did not change with increase in compression wood severity.

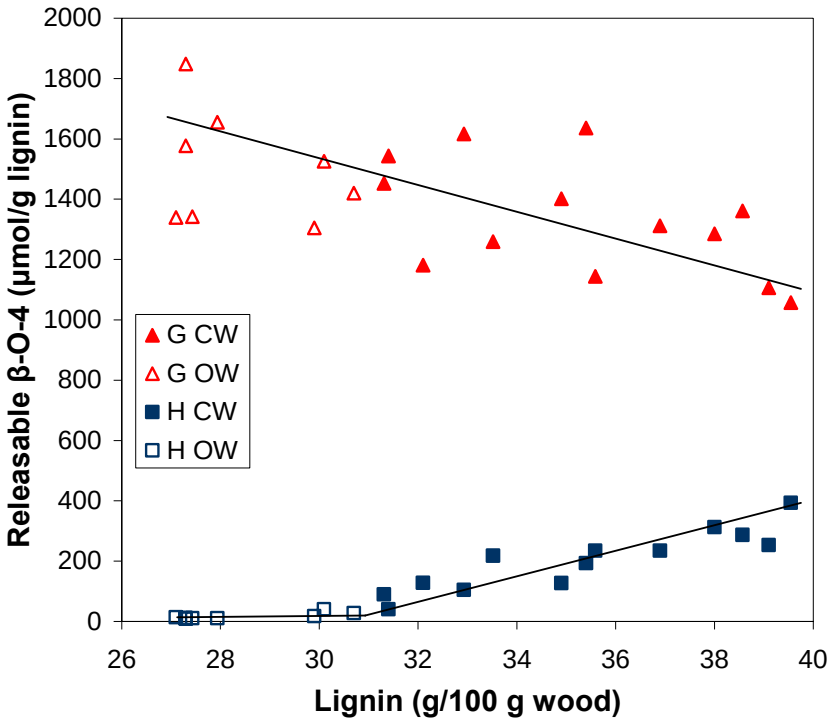


Figure 4.4 Relationships between lignin content and releasable H (R^2 = 0.81) and G (R^2 = 0.28) β-O-4 units in *P. radiata* wood samples.

The H/G ratio as determined by thioacidolysis also showed a linear correlation (R^2 = 0.76) with lignin content (Figure 4.5), but correlation was weaker than for releasable H β-O-4 units alone.

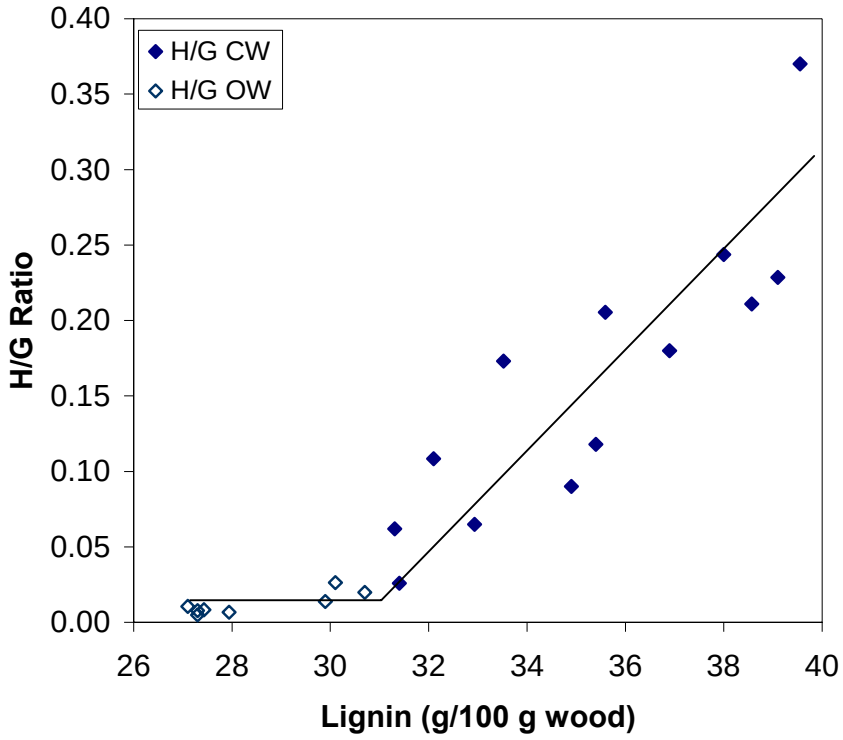
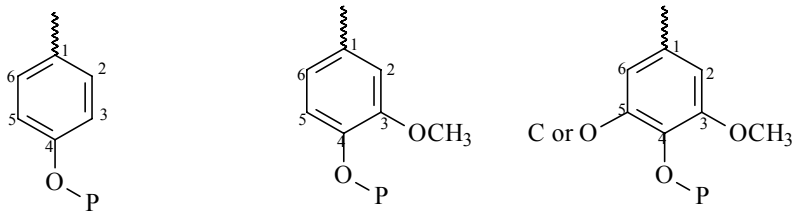


Figure 4.5 Relationship between lignin content and the releasable H/G β-O-4 ratio ($R^2 = 0.76$) in *P. radiata* wood samples.

4.3.2.2 Uncondensed and condensed C9 units

Relationships between amounts of uncondensed H units (**3**) uncondensed G (those unsubstituted or bearing protons at C5) units, (**4**) and condensed guaiacyl units i.e. those substituted at C5 by an O or C (**5**) and lignin content are shown in Figure 4.6. Levels of uncondensed H remained approximately constant in opposite wood, but as compression wood severity increased the levels of uncondensed H units also increased in a linear manner ($R^2 = 0.86$) to a maximum of 18% of the total measured C9 units. There was a tendency for levels of uncondensed G units to decrease with increasing compression wood severity but the correlation was weak ($R^2 = 0.60$). These observations are consistent with results of thioacidolysis, which showed that G β-O-4 units decreased and H β-O-4 increased with increasing compression wood severity. The proportion of condensed G units did not change with compression wood severity. It can be concluded that the level of uncondensed H units is a satisfactory indicator of compression wood severity, while the levels of uncondensed and condensed G units are not suitable.



3 **4** **5**

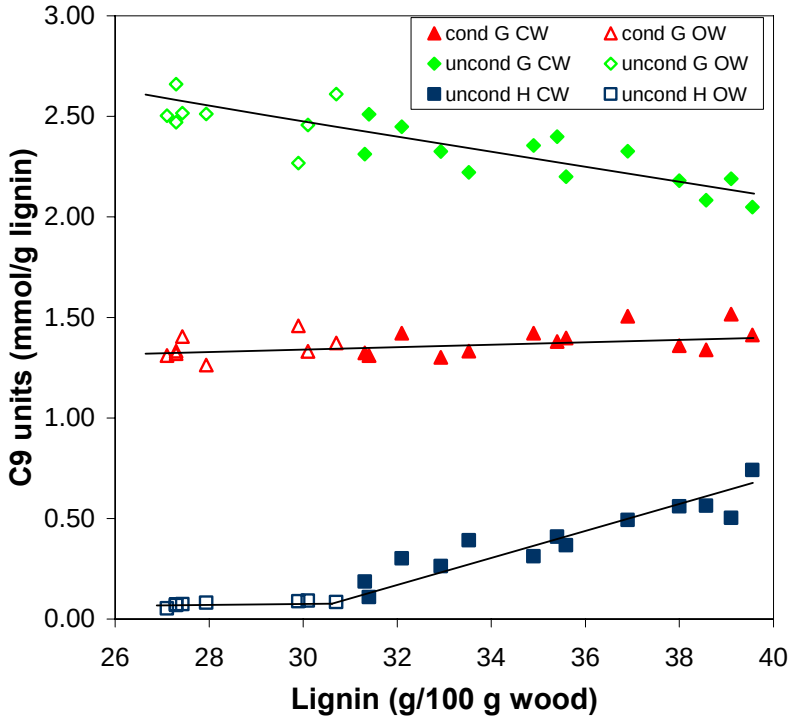


Figure 4.6 Relationships between lignin content of *P. radiata* wood samples and uncondensed H, G and condensed G units as determined by thioacidolysis/^{31}P NMR spectroscopy.

Amounts of releasable H β-ethers, releasable H/G β-ether ratio and uncondensed H C9 units all showed a good correlation with compression wood severity (Table 4.4). All *p*-hydroxyphenyl-related criteria were superior to galactose content and the galactose/glucose ratio as indicators of compression wood severity (Table 4.3). Levels of uncondensed H C9 units were strongly correlated with severity, but the range relative to levels in normal wood was smaller when compared with those of the releasable H β-ethers and H/G β-ether ratio. Releasable H β-ethers and the H/G β-ether ratio which were less closely related to severity than uncondensed H C9 units, satisfied Criteria 2 and 3 i.e. they spanned a larger range relative to levels in normal wood and were less sensitive to the morphological origin of the sample (Table 4.4). The H/G β-ether ratio as measured by thioacidolysis may be the

preferred parameter since it does not require the determination of absolute values. The H/G β-ether ratio can be obtained from a smaller wood sample than that required for analysis of the basic chemical composition and does not require estimation of the lignin content of the sample.

Table 4.4 Performance criteria for releasable H content, H/G ratio and uncondensed H content.

	Releasable H (μmol/g lignin)	H/G	Uncondensed H (mmol/g lignin)
OW_{mean}	18	0.01	0.08
OW_{max}	40	0.03	0.09
OW_{min}	9	0.00	0.05
CW_{max}	393	0.37	0.74
Correlation coefficient (R^2)	0.81	0.76	0.86
$\frac{CW_{max} - OW_{mean}}{OW_{mean}}$	21	36	9
$\frac{CW_{max} - OW_{mean}}{OW_{range}}$	12	12	17

4.3.3 Use of py-GC-MS for detection of H/G ratio in lignin

Pyrolysis gas chromatography mass spectrometry (py-GC-MS) has been used increasingly for the determination of the syringyl/guaiacyl (S/G) ratio (*25*), lignin content (*26*) and H/G ratio in lignin (*27*). Since the H/G β-ether ratio in lignin is a good indicator of compression wood severity we have explored py-GC-MS as a rapid means of detecting the H/G ratio of lignin. Pyrograms of *P. radiata* opposite wood and compression wood are presented in Figure 4.7 and the assignment of peaks is shown in Table 4.5.

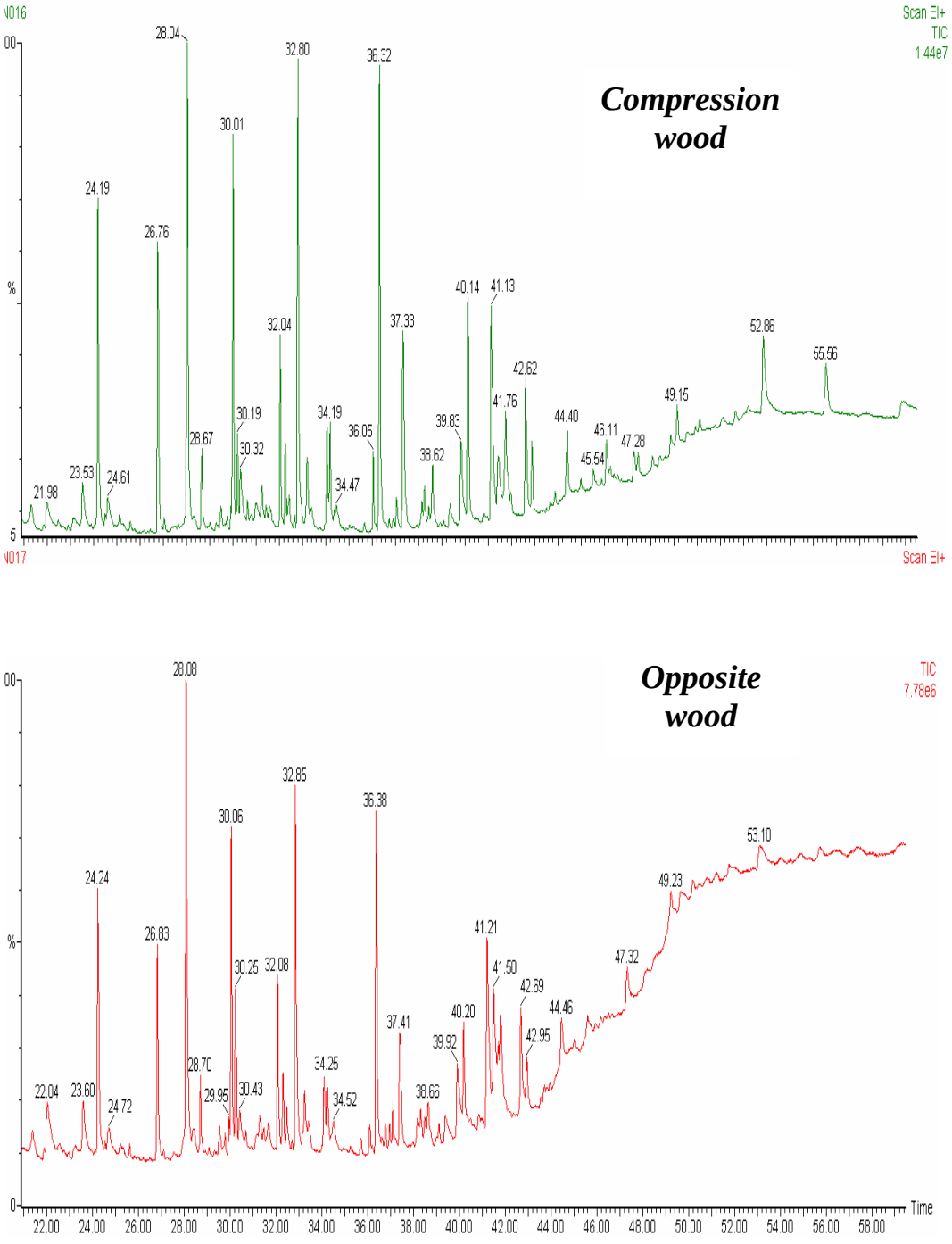


Figure 4.7 Pyrograms of *P. radiata* compression wood and opposite wood (only lignin derived peaks are shown).

Table 4.5 Pyrolysis products of *P. radiata* wood. Degradation products are classified as guaiacyl (G) and *p*-hydroxyphenyl (H) type substances.

Retention time (min)	Mass	Product	Origin
24.2	124	2-Methoxy phenol (guaiacol)	G
26.8	138	4-Methyl guaiacol	G
28.1	94, 107	Phenol and 2-methyl phenol	H
28.7	137	4-Ethyl guaiacol	G
30.1	108	4-Methyl phenol	H
30.2	108	3-Methyl phenol	H
32.1	164	Eugenol	G
32.3	121	4-Ethyl phenol	H
32.8	135	4-Vinyl guaiacol	G
34.2	164	Isoeugenol (*cis*)	G
36.3	164	Isoeugenol (*trans*)	G
37.3	120	Vinyl phenol	H
40.1	134	Propenyl phenol	H
41.1	151	Vanillin	G
41.7	137	Homovanillin	G
42.6	151	Acetovanillone	G
44.4	137	Coniferyl alcohol	G
49.2	137	Dihydroconiferyl alcohol	G
52.9	178	Coniferaldehyde	G

The pyrolysis H/G ratio (calculated as the sum of the areas of all *p*-hydroxyphenyl-derived peak areas divided by the sum of the areas all guaiacyl-derived peaks) was plotted against the lignin content (Figure 4.8). There was no obvious relationship between pyrolysis products and compression wood severity. This is not suprising since the specificity of pyrolysis is low due to random destruction of the phenylpropane units in lignin.

Some researchers relate pyrolysis results to other techniques by analysing large numbers of samples and subjecting data to multivariate analysis (*27*). This approach could not be attempted since the number of samples was limited. The use of an internal standard (*28*) was avoided, not only because it is a complicated method, but also because it was not expected to give different results for the ratio of G and H units.

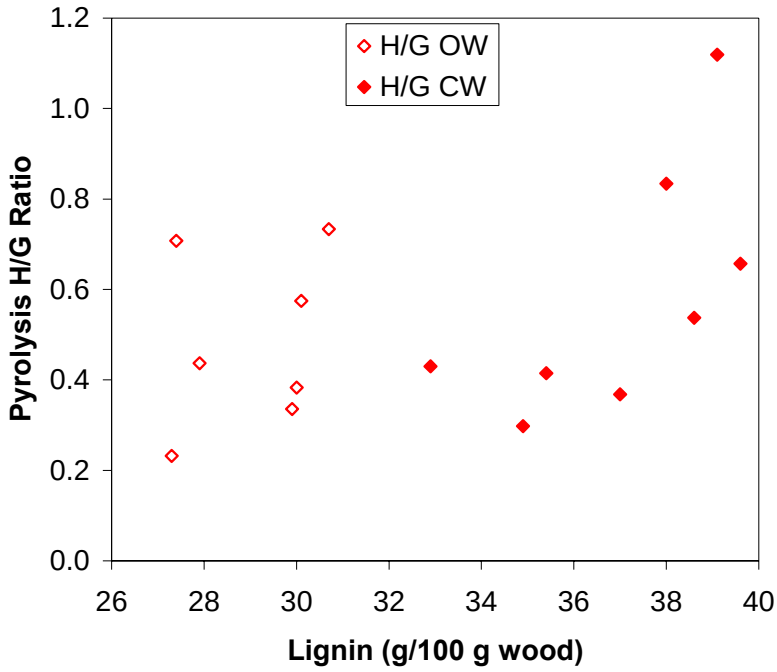


Figure 4.8 Relationship between lignin content and H/G ratio of pyrolysis products derived from *P. radiata* wood.

4.3.4 Releasable monomers and uncondensed units in lignin

Releasable β-ethers determined after thioacidolysis as the monomers **1** and **2** would be analysed as uncondensed G (**4**) and uncondensed H (**3**) C9 units respectively by thioacidolysis/^{31}P NMR spectroscopy. With increasing lignin content the ratio of releasable G β-ether units to uncondensed G units remained constant (Figure 4.9). The releasable G β-ethers constituted approximately 60% of the uncondensed G units in lignin. This is because thioacidolysis only releases β-O-4 ether units that are phenolic or linked at C4 through an ether bond to the α- or β-position of an adjacent unit in the lignin. Other β-O-4 units, e.g. those linked to a second C9 unit by a C-C bond at C-5, are not converted to trithioethers such as **1** and **2**. These β-O-4 units will be analysed as uncondensed units in ^{31}P NMR spectroscopy.

When a similar comparison was carried out for H units it was found that the proportion of releasable H β-ethers to uncondensed H C9 units increased with increasing lignin content (Figure 4.9). This may be attributed to the fact that, unlike G units which are incorporated into the secondary wall in normal and compression wood, H units are mainly incorporated into the compound middle lamella in opposite wood (*29*), and into the secondary wall in compression wood (*30*). Therefore, as the

proportion of secondary wall lignin increases with compression wood formation, the level of releasable H β-ether units would be expected to rise. The low precision of the thioacidolysis-GC method could also account for this observation, since H units are present at very low levels in opposite wood (lignin content 27-31%).

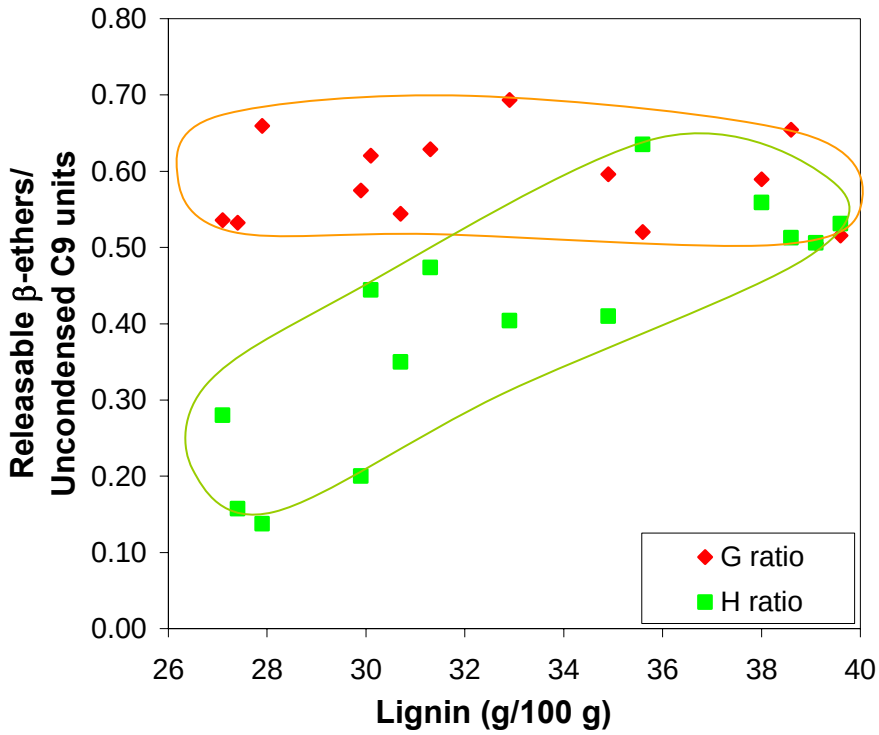


Figure 4.9 Relationships between lignin content and the ratio of releasable β-O-4 units by thioacidolysis to uncondensed C9 units by ^{31}P NMR spectroscopy.

4.4 Conclusions

Chemical differences between compression wood and related opposite wood of *P. radiata* were found to be independent of the morphological origin of the wood sample (stem, branch and young wood).

There was a good agreement between compression wood severity as characterised by fluorescence microscopy and lignin content: the higher the lignin content, the more severe the development of compression wood. The results of this study demonstrate that chemical parameters can be used as an alternative/complementary method to microscopy for quantifying severity.

A number of chemical characteristics were found to be linearly related to compression wood severity. These were: the amount of lignin, and galactose content, the galactose/glucose ratio and the *p*-hydroxyphenyl content of the lignin. Parameters based on the *p*-hydroxyphenyl units in lignin, the H/G β-ether ratio, levels of releasable *p*-hydroxyphenyl β-ether units and uncondensed *p*-hydroxyphenyl C9 units are suitable indicators of compression wood severity. These characteristics spanned a larger range relative to the normal wood levels and were not influenced by the morphological origin of wood samples. Chemical methods for quantifying compression wood severity should focus on the detection and measurement of these parameters.

Future research should be directed towards assessment of the relationship between the H/G releasable β-ether lignin ratio and other quantitative measures of compression wood severity, such as shrinkage. This would demonstrate the usefulness of the H/G β-ether ratio as a compression wood detector.

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CHAPTER 5

5 Isolation and characterisation of galactan from compression wood of *Pinus radiata*

5.1 Introduction

Compression wood has a much higher proportion of galactan (10% or more) than normal wood (*1*). The (1→4)-β-D-galactan of compression wood is usually found to be of the type belonging to the pectic polysaccharides, which are a vaguely-defined entity comprising galacturonan, galactan and arabinan (*2*). Pectic polysaccharides are the main component of the primary cell walls of dicotyledons; for example (1→4)-β-D-galactan has been isolated from cell walls of mung beans (*Vigna radiata* Wilczek) (*3*), tomatoes (*Lycopersicon esculentum* Mill.) (*4*) and flax (*Linum usitatissimum L.*) (*5*).

From the chlorite liquor[1] of compression wood of Norway spruce (*Picea abies*), Bouveng and Meier (*6*) isolated a galactan consisting of linear chains of (1→4)-linked β-D-galactopyranose residues containing 13% uronic acids. This galactan had a degree of polymerisation of 52. Galactan found in Red spruce (*Picea rubens)* consisted of a main chain of 200-300 (1→4)-linked β-D-galactopyranose residues with slight branching through C6 (*2*). Galacturonic acid residues are commonly obtained after hydrolysis of compression wood galactan (*2,6,7*). Jiang and Timell (*7*) reported that 5% of galactose residues carry a single terminal residue of β-D-galacturonic acid attached at C6. A few glucuronic acid residues may also occur as side chains but their mode of attachment is unknown (*2,7*).

Although there appears to be no record of isolation of galactan from compression wood of *Pinus radiata* its structure could be expected to be similar to that of galactans from other softwoods. This chapter describes attempts to isolate and characterise galactan from *P. radiata* compression wood.

[1] The solution resulting from delignification of wood with sodium chlorite.

5.2 Materials and methods

5.2.1 Isolation of galactan from compression wood

A flow diagram showing the steps involved in isolation of galactan from *P. radiata* compression wood is given in Figure 5.1.

5.2.1.1 Delignification of wood and treatment of chlorite liquor

Extractive-free ground (60-mesh) compression wood (10 g) was delignified using sodium chlorite (10 mL, 27%) in the presence of acetate buffer (400 mL) according to the method described by Uprichard (*8*). Reagents were scaled up to match the amount of wood used. After 5 hours the solution was filtered through sintered glass (Porosity 2) under gentle suction. The contents of the filter were rinsed with water (500 mL).

The combined filtrate was dialysed using dialysis tubing with a nominal molecular weight cut-off of 10 kDa. Dialysis was carried out against tap water for 7 days and then against distilled water for 5 days in a continuous system until, the water in the container reached the conductivity of distilled water. The retentate was concentrated to 100 mL *in vacuo* at 50°C and poured slowly into methanol (500 mL), whereupon a white precipitate was formed. The mixture was centrifuged to separate the precipitate which was washed twice with 80% methanol and transferred to a pre-weighed 50 mL round bottom flask using a small amount of petroleum ether. The petroleum ether was removed under reduced pressure and the resultant brown solid dried overnight under vacuum.

The brown solid consisting of a mixture of hemicelluloses was dissolved in water and a saturated solution of barium hydroxide was added over 1 hour. The resulting precipitate was collected, washed with water and purified by reprecipitation with barium hydroxide. After centrifuging, the precipitate was dissolved in a minimum amount of 2M acetic acid and then poured into a methanol:acetone mixture (8:2). The resulting precipitate was washed twice with 80% methanol and transferred to a round bottom flask using a small amount of petroleum ether. The petroleum ether was removed under reduced pressure and the resultant brown solid dried overnight under vacuum.

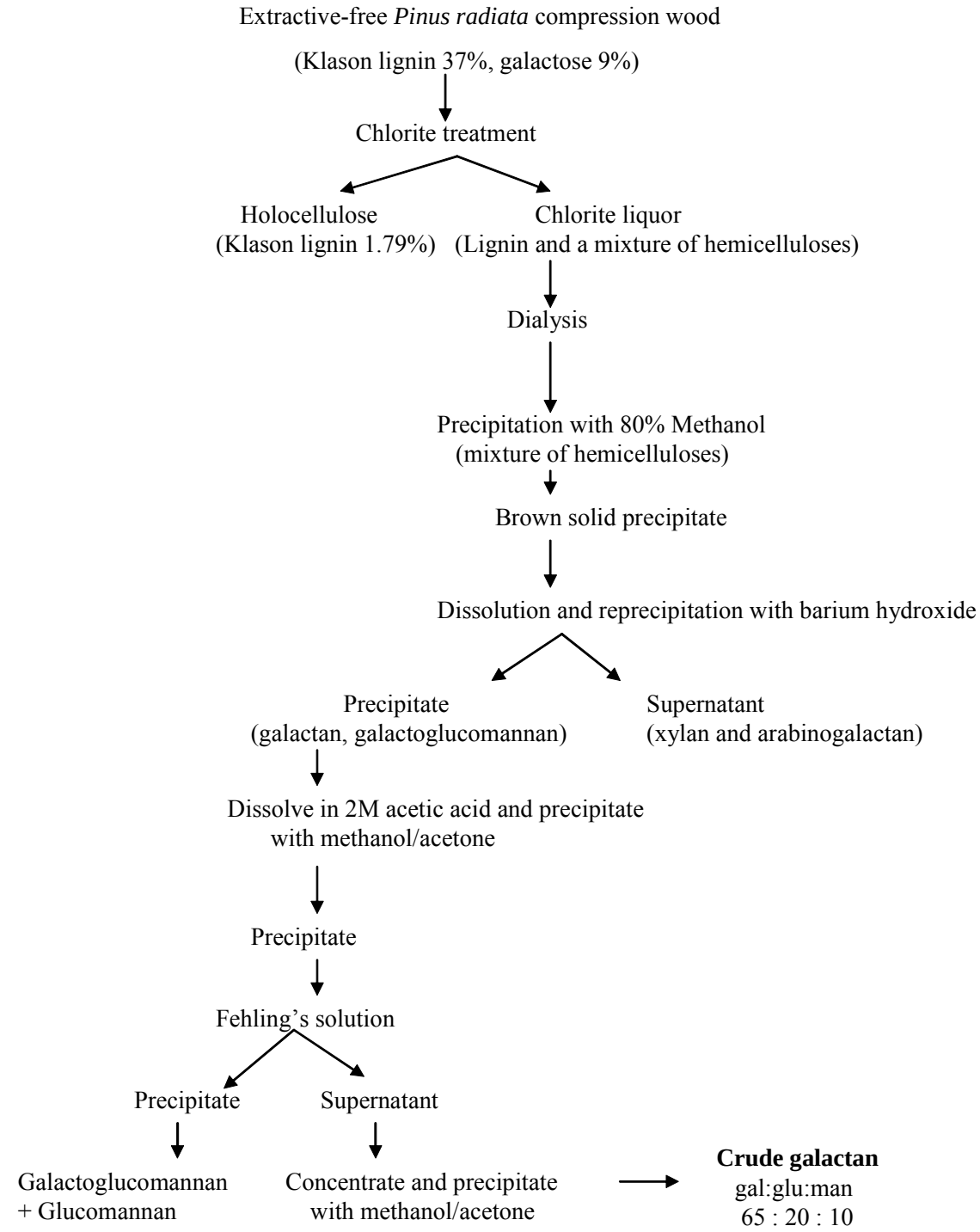

Figure 5.1 Flow diagram showing the steps involved in isolation of galactan from compression wood of *P. radiata*.

The resulting hemicellulose mixture was dissolved in water to a concentration of 5%. Fehling's solution was added slowly with continuous stirring, followed by a small volume of ethanol. The mixture was allowed to stand for 7 hours; the first precipitate appeared after 6 hours. The supernatant which contained the galactan was dialysed

against distilled water for five hours and then treated with Amberlite IR-200 cation-exchange resin (hydrogen form). After concentration, the solution was poured into methanol:acetone. The precipitate was washed twice with 80% methanol and transferred to a round bottom flask, using a small amount of petroleum ether. The petroleum ether was removed under reduced pressure and the resultant brown solid (crude galactan) dried overnight under vacuum (yield 1.9% of wood).

5.2.2 Purification of galactan isolated from compression wood

A solution of crude galactan (30 mg) in water (5 mL) was filtered (Titan syringe filters, nylon pore size 0.45 μm) and loaded onto the column (0.8×65 cm) of DEAE Sephadex-100 (Pharmacia Fine Chemicals) and eluted with MilliQ water (0.5 mL/min) using a Waters 515 HPLC pump and refractive index detection. The column had been calibrated against dextrans having known molecular weight. Appropriate fractions were pooled, concentrated under reduced pressure using a rotary evaporator, and freeze-dried.

5.2.3 Analyses

5.2.3.1 Neutral sugars and uronic acids

Neutral sugars and uronic acids in chlorite liquor were detected by phenol:sulphuric acid (*9*) and *m*-hydroxydiphenyl (*10*) assays respectively. The neutral sugar composition of polysaccharides was determined after hydrolysis with 72% H_2SO_4; monosaccharides were determined by ion chromatography as described by Petterson and Schwandt (*11*). Neutral sugar monomers in isolated galactan were determined quantitatively by formation of alditol acetate derivatives of the glycosyl residues of the polysaccharide as described by Albersheim *et al*. (*12*). They were identified by gas chromatography-mass spectroscopy (GC-MS).

5.2.3.2 Methylation (linkage) analysis

Methylation was performed by the method developed by Hakomori (*13*) using dimsyl anion created with dry DMSO and potassium hydride to ionize the hydroxyl groups. Dry sample (200 μg) was mixed with dry DMSO (100 μL) by stirring overnight in an atmosphere of nitrogen. Dimsyl anion (1 mL) was added to the

mixture and stirred for 6-8 hours. Iodomethane (700 μL) was added to the sample mixture (frozen) and stirred overnight and ultrapure water (1 mL) was added. MeI was bubbled off carefully using a slow stream of nitrogen until the sample mixture was free of MeI. The per-*O*-methylated cell wall polysaccharide mixture was purified by reversed phase chromatography (*14*) using a Sep-Pak C-18 cartridge (Waters Associates, Inc.) and recovered with acetonitrile (2 × 1 mL). The purified material (500 μg) was hydrolyzed with 2M trifluoroacetic acid (TFA, 200 μL), reduced with $NaBD_4$ (15-20 drops of 10 mg/mL solution of $NaBD_4$ in 1M NH_4OH) and acetylated (250 μL of acetic anhydride and 230 μL of concentrated TFA) to give partially-methylated alditol acetates (PMMA). Products (1 μL) were analysed by GC-MS (Supelco SP2330 fused silica column 30 m × 0.25 mm i.d.). The GC conditions were as follows: injector temperature, 250ºC; temperature program 190ºC (2 min) + 10ºC/min to 240º C (10 min). Partially-methylated alditol acetates were identified by using the database of PMMAs available in the Complex Carbohydrate Research Centre website. Http://www.ccrc.uga.edu/specdb/ms/pmaa/pframe.html.

5.2.3.3 Molecular weight determination using the size-exclusion HPLC

Samples (10 mg) were silylated using Tri-Sil reagent (1 mL) prior to HPLC. Silylated samples were dissolved in tetrahydrofuran (~1 mL). Size exclusion chromatography was performed on a HPLC (Viscotek GPC Triple Diode Array 302) equipped with Styrogel HR1, Styrogel HR3 and Styrogel HR4E columns. Elution was carried out at 50°C with tetrahydrofuran at 1 mL/min. The eluent was monitored using a refractive index (RI) detector. Pullulan standards (Polymer Laboratories) were dissolved in tetrahydrofuran and used for molecular weight calibration.

5.2.3.4 NMR spectroscopy

For the NMR experiments 9 mg of the purified galactan was dissolved in 0.75 mL of D_2O (99.97%) and transferred into the NMR tube (5 mm). ^1H spectra were acquired on a Bruker DRX 400 MHz spectrometer at 80°C using a 90° pulse of 6 μs and a relaxation delay of 2 s. The spectral width was 8287 Hz and 32 scans were acquired. The DEPT135 spectra was acquired at 30°C and 135 k scans were acquired.

5.3 Results and discussion

5.3.1 Isolation of galactan from compression wood

The previously described method for isolation of galactan from compression wood (*2,7*) was enhanced by the introduction of an extra separation step using gel chromatography.

Galactan was isolated from the chlorite liquor resulting from delignification of wood rather than from the wood itself. The reason for this is that early workers who attempted isolation of galactan had noticed significant amounts of galactan and galactoglucomannan in the hot chlorite liquor (*2,6,7*). Galactan can be isolated by treating holocellulose (delignified wood meal) with reagents such as hot water, aqueous potassium hydroxide and aqueous sodium bicarbonate. Nevertheless, since a significant proportion of galactan are removed in the chlorite liquor, yields can be very low (*6*). Schreuder *et al*. (*2*) attempted to prevent loss of galactan during preparation of holocelluloses but found that dissolution of a considerable portion of the galactan during delignification was unavoidable. When wood is delignified, gradual removal of the encrusting lignin exposes increasing amounts of polysaccharides to the delignifying agents leading to cleavage of glycosidic bonds.

It is probable that the galactan is present in wood in the form of lignin carbohydrate complexes and that the cleavage of these linkages releases galactan from the complexed form. In the present study, the direct extraction of galactan from compression wood with hot water was found to release less than 1% of galactan. This yield was considered too small to warrant any structural analysis.

The approximate yield from precipitation of the dialysed chlorite liquor with methanol was 5% of the original wood weight. It is interesting to note that compression wood samples having a higher lignin content than normal wood were not difficult to delignify, and that the delignification process was more efficient than for normal wood. The lignin content of holocellulose in compression wood was reduced from 37% to 1.79%, under similar conditions normal wood showed a reduction from 28% to 4.47%. It is possible that the numerous intercellular spaces offered a physical pathway for the chlorite liquor, in which case much of the

compound middle lamella and the primary cell wall layer would be directly exposed to the reagent.

The hemicellulose mixture recovered from the chlorite liquor was purified by complexing twice with barium hydroxide. Barium hydroxide forms insoluble complexes with galactans, galactoglucomannans, and glucomannans, probably by the reaction of barium ions with vicinal *cis*-hydroxyl groups on C2 and C3 of the mannose unit and C3 and C4 of the galactose unit (*15*). The barium complexes are readily soluble in dilute acids and even in water, but are insoluble in organic solvents and in copper ethylene diamine (*15*). The approximate yield of the purified product was 2.1% of the original wood weight. A considerable amount of galactan remained in solution and could not be recovered after the second precipitation. Repeated precipitation led to the loss of material.

Galactoglucomannans forms an insoluble copper (II) complexe with Fehling's solution (*2*). Separation of galactan from galactoglucomannan by precipitation with Fehling's solution did not result in complete precipitation of galactoglucomannan. The resulting product contained 65% galactose, 20% mannose and 10% glucose; uronic acid (~5%) was also detected.

Further purification of the galactan and galactoglucomannan was attempted by gel chromatography using Sephadex-100 gel. The chromatogram is shown in Figure 5.2.

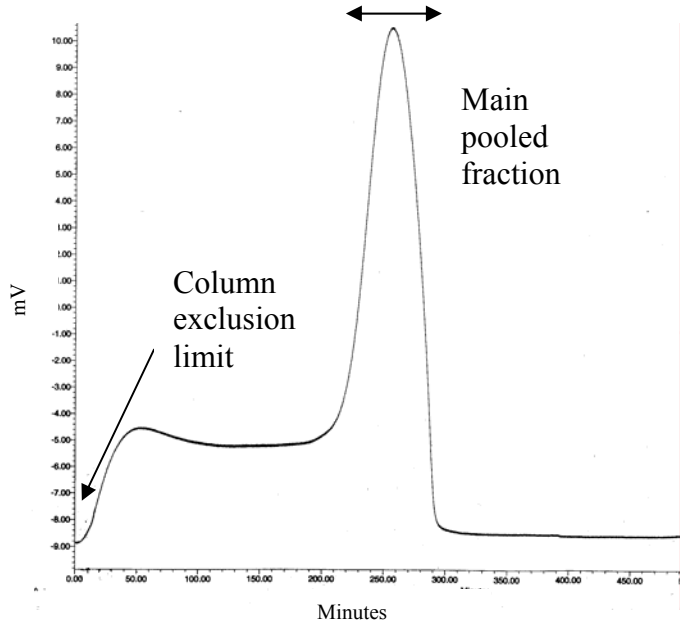

Figure 5.2 Size exclusion chromatogram of galactan isolated from *P. radiata* compression wood.

5.3.2 Characterisation of the main pooled galactan fraction

The main fraction obtained by gel chromatography was analysed to determine the constituents and linkages of the polysaccharide.

5.3.2.1 Glycosyl composition

The galactose content of compression wood can be used as a reasonably accurate measure of the quantity of galactan present (*1*). The molar ratio of sugars from the main pooled fraction was 78% galactose, 13% mannose and 3% glucose conclusively identified by alditol acetate formation and analysis of alditol acetates by the GC-MS. The glycosyl composition indicates that mannose and glucose may be derived from galactoglucomannan present as a contaminant in the galactan fraction. Contamination from galactoglucomannan has been mentioned in several reports (*2,6,16*). For example Iversen *et al*. (*16*), who isolated galactan from *Picea abies,* detected galactose:mannose:glucose in the ratio of 80:15:5. Some reports indicate that xylose is a contaminant (*2*) in crude galactan fractions. Xylose was not found to be present in the current study.

The proportion of galactose residues derived from galactoglucomannan was calculated to be 0.4%, when the sugar composition values reported in the literature for galactoglucomannan of *Pinus radiata* (galactose:glucose:mannose = 0.1:1.0:3.7) (*17*) was used. It is therefore clear that most of the galactose in the hydrolysate was derived from galactan.

5.3.2.2 Methylation analysis

The main fraction was subjected to methylation analysis to determine the glycosyl-linkage composition. One major and a minor peak were identified by the GC-MS chromatogram, which indicated the presence of 1→4-linked hexopyranose. The mass spectrum of the major peak is shown in Figure 5.3. There were no indications of either a branch point or a non-reducing end. A large number of contaminant peaks were observed and it is possible that branch points and non-reducing residue signals were lost in the noise. It was concluded that the polysaccharide consisted largely of unbranched 1→4 linked galactopyranose. The minor GC-MS peak observed probably arose from a 1→4 linked mannopyranose in the galactoglucomannan. These results agree with results from methylation analysis of galactan reported by other workers (*2*,*7*). These workers also detected the 2,3,4,6-tetra-*O*-methylgalactose which originates from non-reducing end groups in the polysaccharide. Although galactan is considered to be difficult to methylate, possibly due to the presence of an axial hydroxyl group at C4, no products indicating undermethylation were found in the present study.

Bouveng and Meier (*6*) also observed that galactan isolated from *Picea abies* could cast into a fairly strong film; this is a feature characteristic of a linear polysaccharide. On the other hand the presence of 5-6 branches have been reported in the galactan fraction directly extracted from *Picea rubens* wood with a DP value of 280 (*2*).

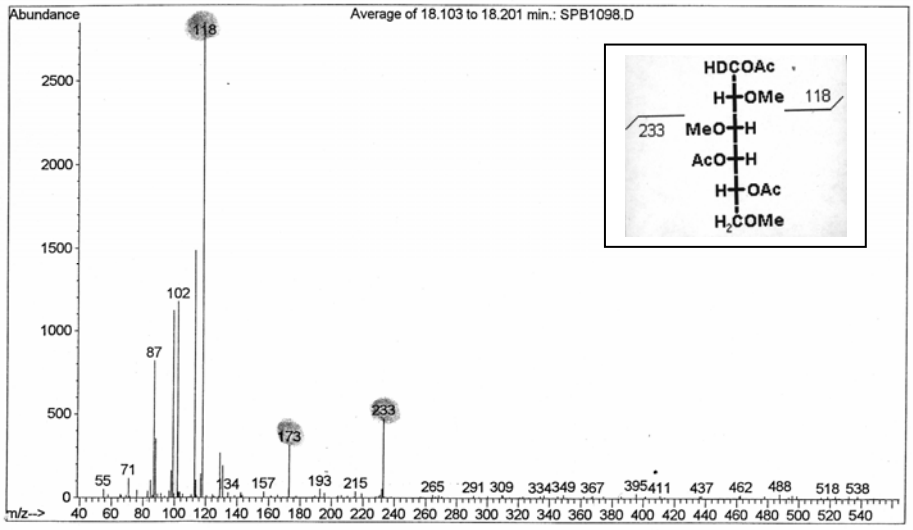

Figure 5.3 Mass spectrum of 1,4,5-tri-*O*-acetyl-1-deuterio-2,3,6-tri-*O*-methyl-galactitol.

5.3.2.3 ^{1}H NMR spectroscopy

In the present study nuclear magnetic resonance spectroscopy was used as an additional structural elucidation tool. The ^{1}H NMR spectrum is shown in Figure 5.4. Due to the complex nature of the isolated fraction the peaks were not well separated. Use of elevated temperature (80°C) improved the spectrum. The doublet at 5.0 ppm ($J_{1,2}$~7 Hz) could be assigned to the anomeric proton of (1→4)-linked β-galactan. The β-anomeric configuration is confirmed by the coupling constant since the α-anomeric configuration would have a coupling constant around 4 Hz in contrast to the 8 Hz characteristics of the β-anomeric configuration. The signal at 4.5 ppm could be assigned to H4 although the peaks due to H2, H3, H5, and H6 were not clearly resolved. ^{1}H NMR chemical shift data reported for a (1→4)-linked β-galactan isolated from mung bean (*Vigna radiata* Wilczek) (*3*), flax (*Linum usitatissimum L.)* (*5*) and spruce (*Picea abies*) (*18*) are shown in Table 5.1.

Table 5.1 ^{1}H NMR chemical shift data for (1→4)-linked β-galactan in D_2O.

Ref	H-1	H-2	H-3	H-4	H-5	H-6
(*3*)	4.20	3.39	3.53	3.81	3.57	3.69
(*5*)	4.68	3.72	3.81	4.21		
(*18*)	4.66	3.69	3.79	4.19	3.73	

A signal corresponding to aromatic protons was present. This might have been an impurity derived from lignin degradation products.

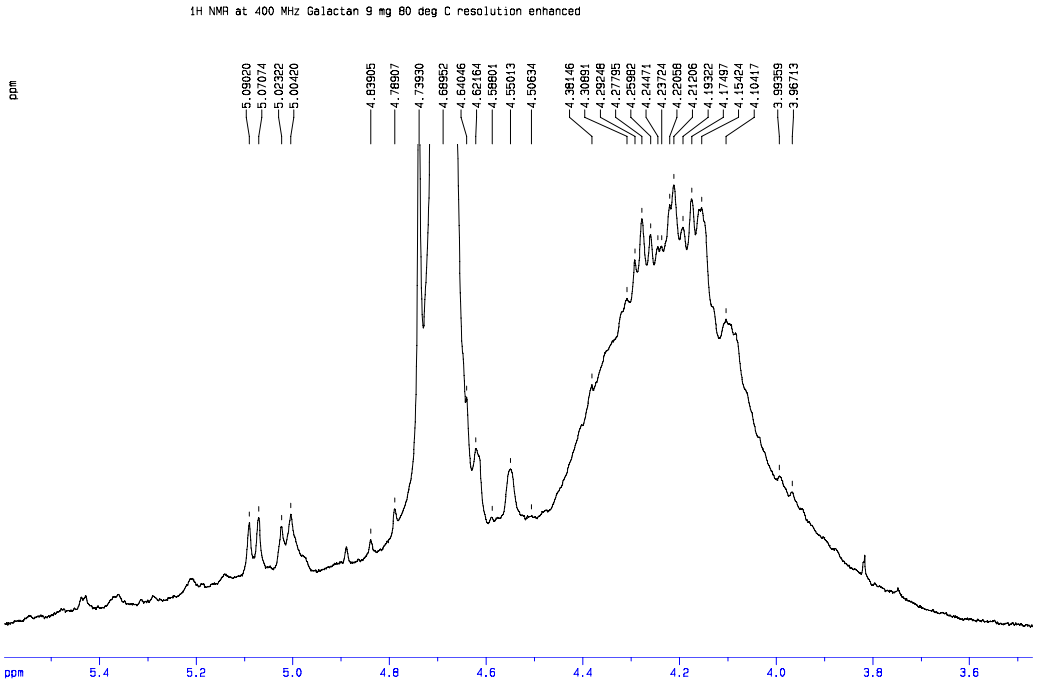

Figure 5.4 ^{1}H NMR spectrum of galactan isolated from *Pinus radiata* compression wood.

5.3.2.4 ^{13}C NMR spectroscopy

The DEPT135 spectrum contained 6 strong signals at 106.3, 79.6, 76.5, 75.3, 73.8 and 62.7 ppm, the latter being inverted with respect to the other signals. A few weaker signals were also observed (Figure 5.5).

The peaks were assigned by comparison with chemical shift data reported in the literature (Table 5.2). The signal at 106.3 ppm was considered to be due to C1 of β-D-galactopyranose in a glycosidic linkage. ^{13}C NMR data reported for oligosaccharides assign 101.3 ppm for C-1 of α-Gal(1→4) while β-Gal(1→4) is

104.2 ppm (*19*). This was additional confirmation of evidence of a β-linkage obtained from the ^{1}H NMR spectra. The inverted signal at 62.7 ppm is assigned to C6. Despite the fact that the spectrum was noisy, there were no other down-field shifted extra C6 signals, which would be expected if branching occurred at C6. Signals at 73.8, 75.3, 79.6 and 76.5 were assigned to C2, C3, C4 and C5 respectively. There was a good agreement between the chemical shifts obtained in the present study and values reported in the literature (Table 5.2).

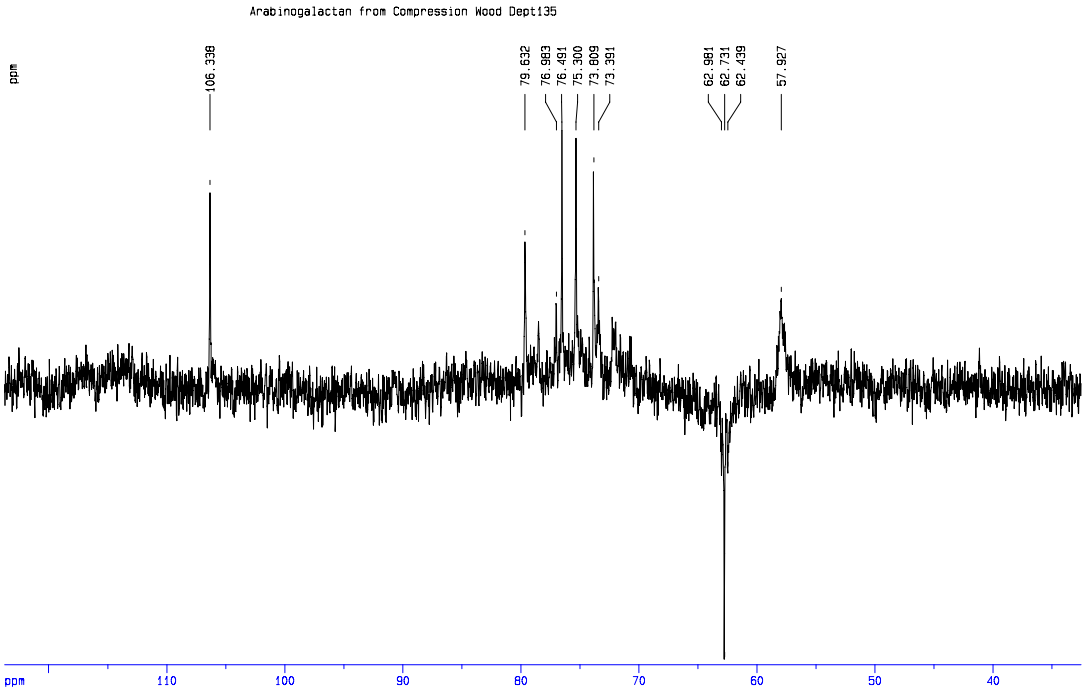

Figure 5.5 ^{13}C DEPT 135 spectrum of galactan isolated from *Pinus radiata* compression wood.

Table 5.2 ^{13}C NMR chemical shift data for (1→4)-linked β-galactan in D_2O. Data from the other studies are shown for comparison.

Reference	C-1	C-2	C-3	C-4	C-5	C-6
	106.3	73.8	75.3	79.6	76.5	62.7
(*3*)	105.8	73.3	74.8	79.1	75.95	62.2
(*5*)	105.8	73.3	74.8	79.1	75.95	62.2
(*20*)	106.7	73.1	74.6	78.9	75.81	62.1
(*18*)	105.4	72.8	74.3	78.7	75.60	-

Information obtained by NMR spectroscopy was consistent with results from methylation analysis and showed that the predominant component of the isolated galactan fraction was (1→4)-linked β-D-galactopyranose. There was no direct indication of branching at C-6. Most of the ring carbons appear in the region 70-80 ppm and thus it was difficult to interpret this part of the spectra.

5.3.2.5 Molar Mass

According to SEC determinations, the average molecular weight of the main fraction was around 60 kDa, a DP of approximately 390. This value should be viewed with caution since contaminants were present in the fraction. The sample was silylated prior to analysis of the molecular weight and the reported figure is based on the assumption that all hydroxyl groups in the polysaccharide were silylated. A wide variation in DP figures (52-300) for galactan isolated from spruce species has been reported in the literature (*2,6*). It is suggested (*1,2*) that the DP of galactan should be 200-300. This value is higher than that of other hemicelluloses such as galactoglucomannan which has a DP of approximately 120 (*18*).

5.4 Conclusions

Galactan was isolated with a purity of approximately 80% galactose from *Pinus radiata* compression wood. Structural investigation by methylation analysis and NMR spectroscopy revealed that this galactan was largely comprised of (1→4)-linked β-D-galactopyranose residues. No evidence was found of the presence of any branched structures.

5.5 References

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CHAPTER 6

6 Lignin structure of *Pinus radiata* thermomechanical pulp fractions and callus tissue

6.1 Introduction

Chapters 3 and 4 described lignin characterisation techniques that were used to study compression wood samples. This Chapter describes the use of these techniques to investigate the structure of lignin in a number of non-compression wood samples. Specifically, the middle lamella- and secondary wall-enriched thermomechanical pulp (TMP) fractions from normal wood.

In recent years, attention has focused on the variation of lignin distribution and structure in the different layers of the cell wall. According to Sovari *et al*. (*1*), middle lamella lignin usually has a higher free phenolic content, a lower methoxyl content and a higher proportion of condensed units than lignin in the secondary wall. Nevertheless, there is considerable variability among reported descriptions of the characteristics of middle lamella and secondary wall lignin (see Section 2.6).

Several approaches have been used in the fractionation of compound middle lamella and secondary cell wall material from wood and fibre. Early workers used density-gradient techniques (*1*) because lignin has a lower density than carbohydrates. Lignin-rich material such as the middle lamella will float, and low-lignin content material such as the secondary cell wall will sink, if a solvent with appropriate density is chosen (*2*). This technique is based on the assumption that middle lamella material is the only lignin-rich component of wood. It is now known that compression wood and ray cells are also rich in lignin (*3,4*).

A more efficient approach to the characterisation of middle lamella and secondary wall lignins is the fractionation of mechanical pulp to yield fibre enriched with secondary cell wall material and a fines fraction enriched with middle lamella

material (*5-7*). This involves passing of a pulp suspension through a series of sieves with decreasing mesh size in order to separate components by particle size and purification of these components by sedimentation. This method is quick and simple and yields material in a reasonably pure condition.

Also described in this chapter is characterisation of lignin found in the callus tissues of *Pinus radiata,* which is of interest to other researchers of Scion Cell Wall Biotechnology Centre.

6.2 Materials and methods

6.2.1 Middle lamella and secondary cell wall material

Fibres and fines fractions prepared by a thermomechanical process were obtained from Dr John Richardson, Ensis Papro. The wood sample was taken from 12 year-old *P. radiata* obtained as thinnings from Compartment 649, Lake Taupo Forest. The thermomechanical pulp with High freeness[1] (~650 Canadian Standard of Freeness, CSF) was a product of the Ensis Papro Fibre Processing pilot plant (Figure 6.1) which incorporated a Jyhla SD 52/36, pressurised, 900 mm, 1250 kW, 1500 rpm single disc refiner.

The high freeness pulp had been produced from wood chips by a single refining procedure. This was carried out under the following target process conditions:

Atmospheric chip pre-steaming:	80°C
Chip preheating:	3 minutes @ 90°C
Production rate:	11 o.d. kg/min
Discharge consistency:	35%
Refiner inlet/outlet pressures:	100/120 kPa
Primary-stage energy application:	1300 kWh/o.d.t

[1] The rate at which water will drain through the pulp. Refining decreases the pulp freeness, consequently high freeness pulp is produced with low refining energy.

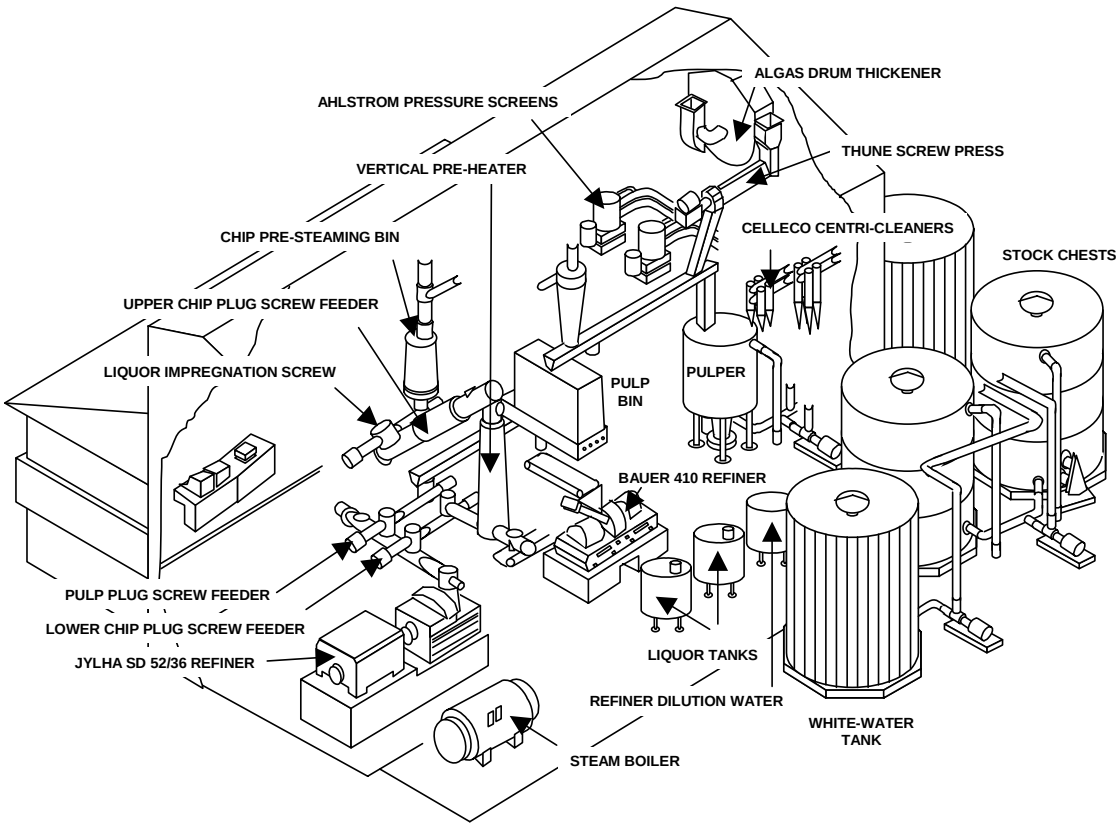


Figure 6.1 The Ensis Papro Fibre Processing Plant (Courtesy of Dr John Richardson, Ensis Papro).

Fines had been obtained from the high freeness pulp by screening the product of P2-S2 stage (Figure 6.2) on a 100 µm bow screen. This ensured that any remaining fibre was removed from the fines.

Further purification of the P2 fines received from Dr Richardson into fibrillar and flake-like material was attempted by overnight sedimentation following the method described by Kangas *et al*. (*8*). No floating fibrillar material was found, possibly because this was primary stage pulp. The clear supernatant was discarded and the sediment diluted to 20 L. The resulting suspension was allowed to settle overnight, and the settled fines were used for analysis. The material was examined by scanning electron microscopy and shown to contain no ray cells or fibrillar fibres. The fibre fraction (S2) was analysed as received.

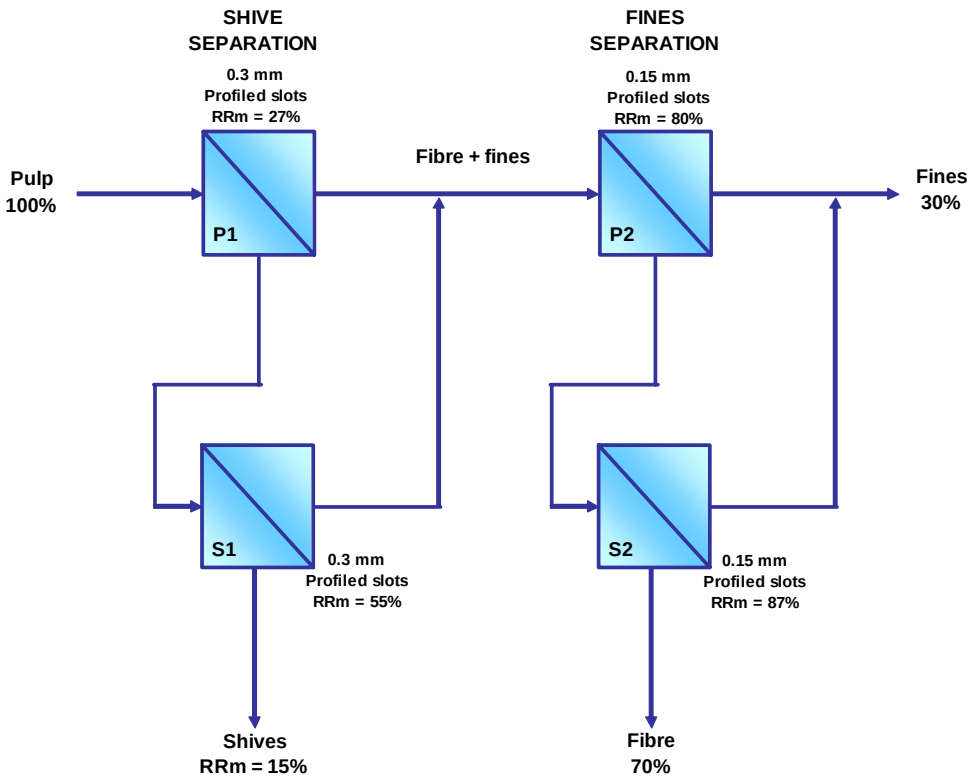


Figure 6.2 Processing configuration for removal of shives from fibre and separation of fines from fibre.

6.2.2 Callus tissue

Unmodified callus tissue was provided by Dr Ralf Möller, Scion Cell Wall Biotechnology Centre while Dr Armin Wagner, Scion Cell Wall Biotechnology Centre provided the transgenic callus tissues. Details of the preparation of callus tissue are described in (*9*). The thioglycolic acid lignin and acetyl bromide lignin content of the callus tissues were determined by Scion Cell Wall Biotechnology Centre using methods described in (*10,11*).

6.2.3 Klason lignin and sugars

Air-dried unscreened TMP, fibre and fines fractions were treated with dichloromethane to remove extractive materials, and then ground to pass a 60 mesh seive. Klason and acid-soluble lignin content were determined according to Tappi Standards T222 om-88 and UM 250 respectively. Monomeric carbohydrate content of the filtrates from Klason lignin determination was determined by ion chromatography (*12*). Results were expressed as anhydro sugar units.

6.2.4 Scanning electron microscopy

A small amount of air-dried sample was coated with chromium in a sputtering device. Images were obtained from a JEOL 6700 field emission scanning electron microscope operated at an accelerating voltage of 10 kV.

6.2.5 Releasable β-O-4 units

Releasable β-O-4 units were determined by the method described in Chapter 3.

6.2.6 Thioacidolysis/^{31}P NMR spectroscopy

Thioacidolysis/^{31}P NMR spectroscopy was performed by the method described in Chapter 3.

6.2.7 Size exclusion chromatography

Size exclusion chromatography was performed by the method described in Chapter 3.

6.2.8 Py-GC-MS

Pyrolysis-GC-MS was performed as described in Chapter 4.

6.3 Results and discussion

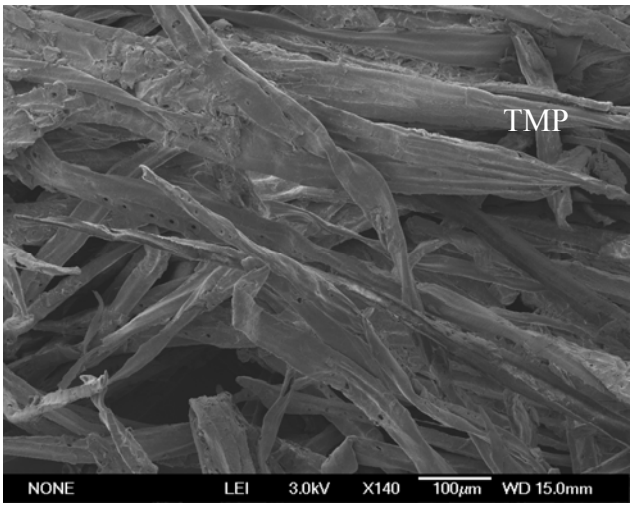
6.3.1 Characterisation of lignin in middle lamella and secondary wall

6.3.1.1 Scanning electron microscopy (SEM)

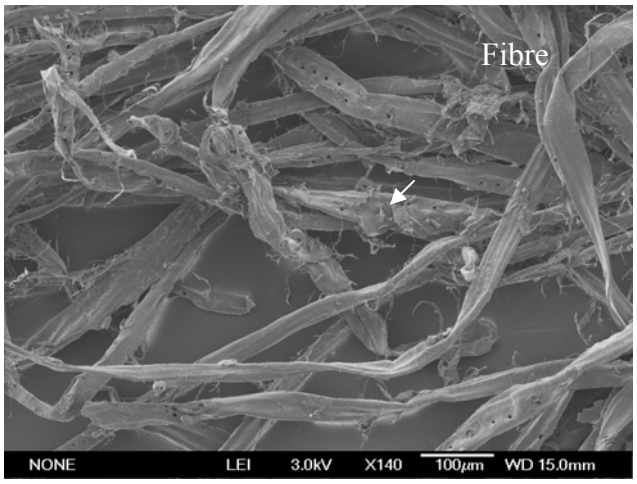
Light microscope with a polariser was used to confirm that the fines fraction of the TMP pulp consisted of middle lamella material while the fibre fraction contained secondary cell wall material. Any birefringent fibre, will change the polarization of the light beam and permit the light to pass through the analyser to the eye. Fines fraction was not visible under polarised light showing that it has originated from the middle lamella mostly composed of lignin which are non birefringent. In contrast, fibre fraction appeared as bright specks against a dark background since it is mainly composed of cellulose-rich material which is birefringent under polarized light due to

the ordered fibrillar structure. This observation confirmed that the separation had been effective. Fractions were further examined by SEM and images are shown in Figure 6.3.

(i)



(ii)



(iii)

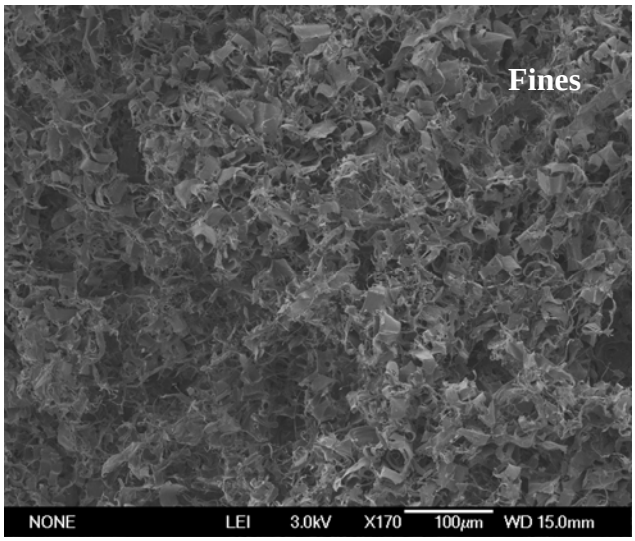


Figure 6.3 Scanning electron micrographs of (i) thermomechanical pulp samples as received; (ii) the separated fibre material and (iii) the separated fines. The arrow indicates a "peeled" fibre.

The fibre fraction consisted mainly of whole fibres having an orderly fibrillar structure plus some "peeled" or broken fibres (indicated by the arrow in Figure 6.3). Some flake-like material stuck to fibres. Small amounts of middle lamella were observed on the fibre surface.

The fines were small, thin ribbon-like flakes. No whole fibres were present. The ribbon-like material may have been produced by peeling of the compound middle lamella during TMP processing. Ray cells were not present. The SEM characterisation of the different fractions showed good agreement with published results (*5*,*13*).

6.3.1.2 Basic chemical composition and lignin structure characterisation

The lignin content of the fines fraction was considerably higher than that of the fibre fraction (Table 6.1). The fines fraction contained a greater proportion of the sugars arabinose, galactose and a lower proportion of mannose and glucose than the fibre fraction. These observations are generally consistent with other reports on the cell wall compositions of the two TMP fractions (*1*,*7*,*14*). Nevertheless, there is some discrepancy in the reported levels of xylose in the fines fraction. While some studies have reported significantly higher levels of xylose in the fines fraction (*1*,*7*) others (*14*) do not report such high levels similar to the results reported here. The reason for this discrepancy is unclear, although species difference might be a reason.

Thioacidolysis was used to determine how the levels of releasable guaiacyl (G) and *p*-hydroxyphenyl (H) β-O-4 linkages in lignin changed across the cell walls. The total yield of thioacidolysis monomers gives a measure of the proportion of lignin units involved in labile β-O-4 bonds (Table 6.1). The yield of releasable β-ethers from the middle-lamella rich fines fraction was lower than from the secondary wall material, indicating that the proportion of resistant inter-unit bonds in the middle lamella is higher than that in the secondary cell wall. In other words, middle lamella contains a more condensed lignin with many inter-unit linkages. This observation agrees with some (*1*,*6*,*7*) but not all of the reports in the literature (*15*). The content of releasable β-ethers in the fibre fraction was similar to that in unscreened TMP and whole wood, indicating that lignin in these three materials was similarly condensed.

Table 6.1 Lignin, monosaccharides and releasable β-ether contents.

	Component (g/100 g extractive-free material)						Releasable β-O-4 units (μmol/g lignin)	
	Lignin	Ara	Gal	Glc	Xyl	Man	G	H
Wood	28.1	1.7	2.4	43.2	6.5	10.8	1701	<9
TMP	28.2	1.8	2.6	44.6	6.5	11.1	1624	<9
Fibre	26.6	1.6	2.3	47.6	6.1	12.1	1688	<9
Fines	40.0	2.7	3.9	31.7	6.7	6.6	1024	<9
LSD[1]	1.1	0.2	0.3	3.1	0.9	1.3	237	6

[1] LSD, least significance difference for $p = 0.05$ (Appendix A).

There was no statistically-significant difference between the amounts of releasable β-ethers in wood and in unscreened TMP. Difference between the releasable β-ethers of wood and TMP (1682 *vs.* 1498 μmol/g lignin) has been reported before (*16*) but statistical significance of this difference was not indicated (*7*).

Releasable H β-O-4 units were present in trace amounts in the wood, unscreened TMP, fibre and fines fractions. This indicates that the middle lamella does not contain elevated levels of releasable H units, contrary to some literature reports of higher levels of p-hydroxyphenyl units in the middle lamella lignin (*17*,*18*). Westermark (*6*), Adams (*15*) and Christiernin *et al*. (*19*) have also reported the absence of uncondensed H units or very low levels of uncondensed H units in middle lamella material.

Size exclusion chromatograms of the thioacidolysis products of whole wood, unscreened TMP, fibre and fines are shown in Figure 6.4.

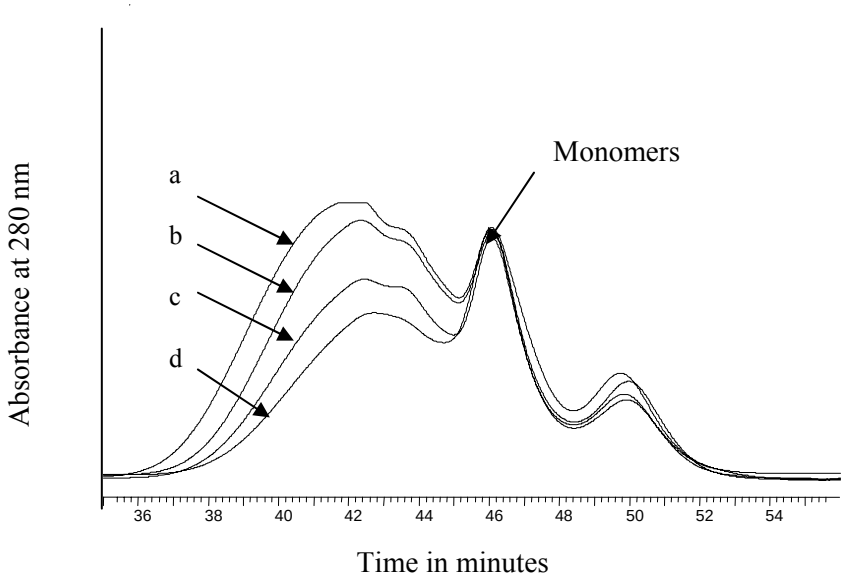


Figure 6.4 Size exclusion chromatograms of thioacidolysis products from (a) Fines; (b) TMP; (c) Fibre and (d) Wood. Peaks eluting after the monomers are not lignin-related. The chromatographs are normalised with respect to the monomer peak.

The area of peak corresponding to the oligomers was larger for the fines fraction than for wood, unscreened TMP or fibre. This observation, together with supporting thioacidolysis data which implies that middle lamella lignin is more condensed than the secondary wall lignin. These results confirm the findings of several studies on the nature of middle lamella lignin (*7,19*).

Results of ^{31}P NMR analysis of the lignin thioacidolysis products phosphitylated with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane are shown in Table 6.2.

Table 6.2 Thioacidolysis/^{31}P NMR spectroscopy results.

	C9 units (mmol/g lignin)							%
Sample	β-5	4-O-5	5-5	Cond. G	Uncond. G	Uncond. H	Total	Cond. G
Wood	0.42	0.23	0.63	1.28	2.65	0.01	3.95	33
TMP	0.44	0.26	0.72	1.42	2.41	0.02	3.86	37
Fibre	0.45	0.28	0.70	1.43	2.55	0.03	4.01	36
Fines	0.43	0.26	0.88	1.57	2.38	0.01	3.97	40
LSD[1]	0.08	0.07	0.12	0.18	0.16	0.02	-	-

[1] LSD, least significance difference for $p = 0.05$ (Appendix A).

The middle lamella-rich fines fraction showed elevated levels of 5-5 condensed structures when compared with the secondary cell wall-rich fibre fraction. As a result, the proportion of condensed G units was highest (40%) in the fines fraction. This observation is consistent with the thioacidolysis results, in which the fines fraction yielded the lowest level of releasable G β-O-4 units. The differences in the releasable G β-O-4 units between the fines and fibre fractions were far greater than the differences existing in condensed G units. The reason for this observation is not clear but it needs to be borne in mind that the releasable β-ethers are only a small portion (~25 %) of the C9 units. Levels of uncondensed H units were very low for all samples, which was again consistent with the thioacidolysis results.

The difference between the wood and unscreened TMP samples in terms of the proportion of condensed guaiacyl appeared to be due to a decrease in the amount of uncondensed units that could have occurred during the pulping process (*16*).

6.3.2 Characterisation of lignin in Pinus radiata callus tissue

6.3.2.1 Unmodified callus tissue

Biochemical and enzymatic studies of lignin biosynthesis have been investigated in plant cell cultures. Lignin produced in undifferentiated cell suspension cultures devoid of secondary cell wall material is often used for investigation of primary cell wall lignin structure (*20,21*). Recently, Möller *et al*. (*22*) reported a *P. radiata* cell culture system in which differentiation of cells with lignified secondary walls had been induced. The lignin produced in these callus cultures is reasonably pure and provides an interesting insight into the mechanism of lignin biosynthesis. In the present study, the structure of lignin in lignified secondary walls of cell cultures was compared with that of *P. radiata* wood using thioacidolysis (*9*).

Thioacidolysis of a callus tissue produced a significantly higher yield of releasable β-ethers. The H\G ratio was very similar to that of wood (Table 6.3). This indicates that callus lignin is almost devoid of condensed structures. The callus samples used in the study contained 18% lignin. This was much less than normal wood because it contained only 15% of lignified tracheary elements, the rest of the sample consisting of undifferentiated cells. Due to the limited amount of material available thioglycolic acid lignin rather than Klason lignin was determined.

Table 6.3 Lignin and releasable β-ether contents of *P. radiata* callus sample and wood.

Sample	Lignin (g/100 g)	µmol/g lignin G	H	H\G
Callus	18[1]	3349	137	0.04
P. radiata wood	28.9[2]	1577	12	0.01

[1] Thioglycolic acid lignin
[2] Klason lignin

Size exclusion chromatograms (Figure 6.5) indicate that the proportion of dimers, trimers and tetramers in thioacidolysis products from callus tissue was significantly lower than those from wood. This agrees with the significantly higher yields of releasable β-ethers obtained from callus tissue lignin.

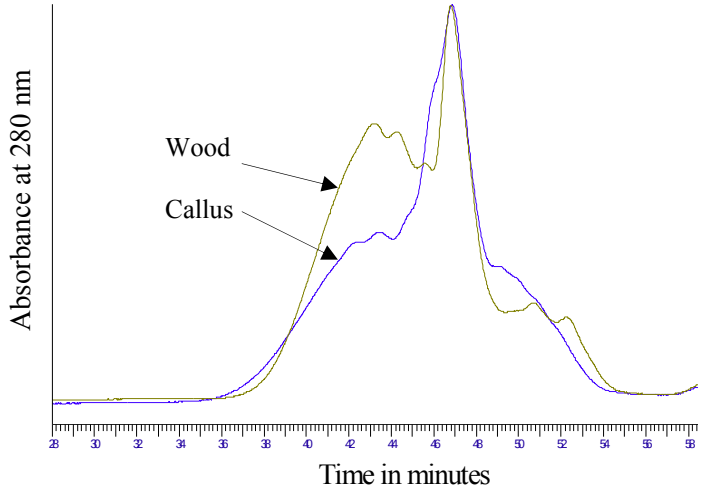


Figure 6.5 Size exclusion chromatograms of thioacidolysis products from *P. radiata* callus and wood.

Results of ^{31}P NMR analysis of thioacidolysis products from the callus tissue and wood are shown in Table 6.4. The callus lignin had fewer condensed G units, greater amounts of uncondensed G structures and fewer β-5 condensed structures.

Table 6.4 Thioacidolysis/^{31}P NMR spectroscopy results.

Sample	C9 units (mmol/g lignin)							
	β-5	4-O-5	5-5	Cond. G	Uncond G	Uncond H	Total	% Cond G
Callus	0.24	0.20	0.59	1.03	2.91	0.20	4.22	26
Wood	0.44	0.22	0.67	1.33	2.47	0.07	3.88	35

6.3.2.2 Transgenic callus tissues

Transgenic callus samples produced by down-regulating the gene controlling the enzyme HCT (hydroxycinnamoyl CoA) in the lignin biosynthetic pathway (see Figure 2.5) were analysed by thioacidolysis, to detect levels of releasable H and G β-ethers in the callus lignin (Table 6.5).

Table 6.5 Lignin and releasable β-ether content of *P. radiata* transgenic callus and wood.

Sample	Lignin (g/100 g)	μmol/g lignin G+H	G	H	H/G ratio
Control callus	28.9[1]	1886	1886	Trace	-
Transgenic callus 1	16.7[1]	791	560	232	0.41
Transgenic callus 2	23.4[1]	1136	769	340	0.44
Transgenic callus 3	18.2[1]	1335	1149	189	0.16
Normal wood	28.9[2]	1589	1577	12	0.01
Severe compression wood	39.6[2]	1450	1057	393	0.37

[1] Acetyl bromide lignin
[2] Klason lignin

Down-regulation of the genes involved in the phenylpropanoid pathway altered the distribution of primary lignin monomers. In transgenic callus Sample 1 the lignin accumulation was 40% lower than that in the control callus sample. The releasable β-O-4 yield was ~50% lower than that from control callus, indicating that the lignin was more condensed. Releasable H β-O-4 units were 29% of the total β-ether yield in Transgenic callus Sample 1 in comparison to control callus where H units were only present in traces. These results were comparable with severe compression wood from

branch where the levels of releasable H β-O-4 units were 27% of the total releasable β-ethers (Table 6.5).

Pyrograms supplied by Dr Armin Wagner, Scion, Cell Wall Biotechnology Centre are shown in Figure 6.6 and major pyrolysis products are listed in Table 6.6.

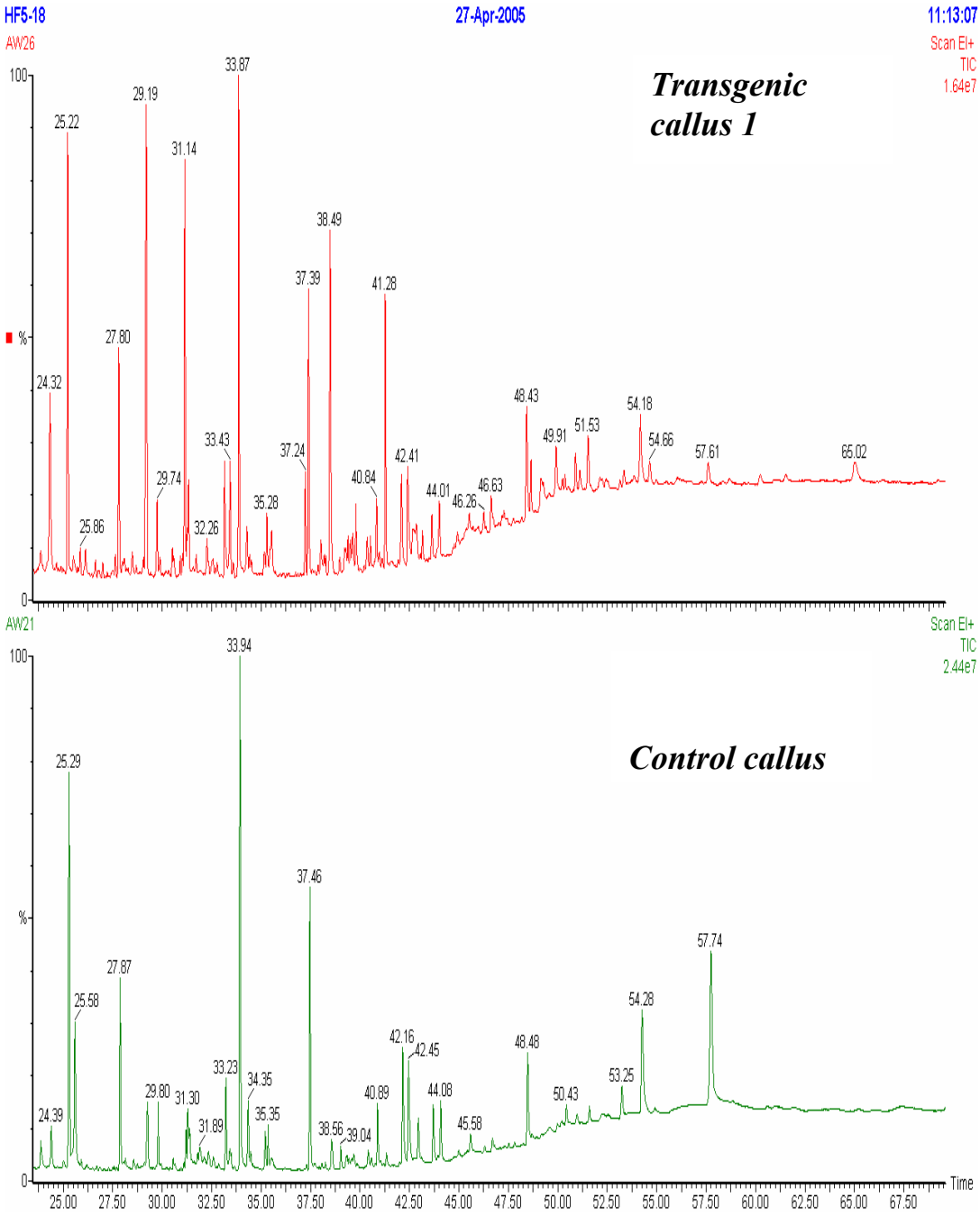


Figure 6.6 Pyrograms for transgenic callus and control samples (only lignin derived peaks are shown).

Table 6.6 Main pyrolysis products of callus tissues.

Retention time (min)	Mass	Product	Origin
25.2	124	2-Methoxy phenol (guaiacol)	G
27.8	138	4-Methyl guaiacol	G
29.2	94 & 107	Phenol and 2-methyl phenol	H
29.7	137	4-Ethyl-guaiacol	G
31.1	108	4-Methyl phenol	H
31.3	108	3-Methyl phenol	H
33.2	164	Eugenol	G
33.4	121	4-Ethyl phenol	H
33.9	135	4-Vinyl guaiacol	G
35.3	164	Isoeugenol (*cis*)	G
37.4	164	Isoeugenol (*trans*)	G
38.5	120	Vinyl phenol	H
41.3	134	Allyl phenol	H
42.4	151	Vanillin	G

Many of the H-derived peaks (phenol, 3-methyl phenol, 4-methyl phenol, vinyl phenol) were present in lower amounts in the control callus than in the transgenic callus and the allyl phenol signal was completely absent from the control callus. The H/G ratio of callus lignin was calculated from the py-GC-MS results using the relative sum of peak areas of all H-derived peaks (phenol, 3-methyl phenol, 4-methyl phenol, vinyl phenol and allyl phenol) and G-derived peaks (guaiacol, 4-methyl guaiacol, 4-vinyl guaiacol, 4-ethyl guaiacol, eugenol, isoeugenol and vanillin). The higher level of H units in the lignin of transgenic callus Sample 1 was further confirmed by its higher H/G ratio (1.0) when compared with that of control callus (0.27).

6.4 Conclusions

From the study of lignin in middle lamella and secondary cell wall fractions of *Pinus radiata* it can be concluded that middle lamella material has a higher lignin content, a lower amount of releasable β-ethers and a more condensed structure. Levels of releasable *p*-hydroxyphenyl β-ethers units were not higher in middle lamella lignin.

6.5 References

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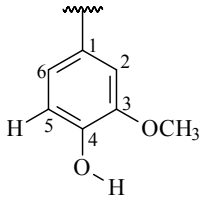
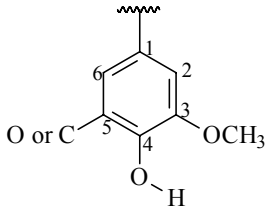
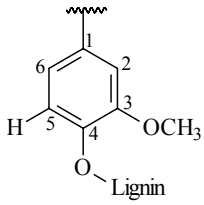
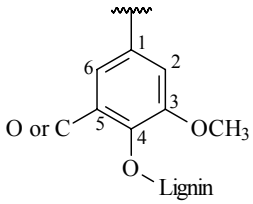
CHAPTER 7

7 A new method for determination of the degree of condensation in the phenolic and etherified C9 units in *in situ* lignin

7.1 Introduction

Softwood lignin is a three-dimensional polymer formed through the coupling of phenyl propane monomers. Due to the complex nature of the coupling reaction, a number of different inter-unit linkages are formed. The resulting lignin contains both uncondensed (protonated at C5) and condensed (bonded through C5 to other C9 units) C9 units. These condensed and uncondensed units may be either phenolic or etherified (Table 7.1). Phenolic uncondensed units in lignin play a prominent role in lignin reactivity (*1*,*2*), and it is therefore important to be able to determine the degree of condensation of phenolic and etherified C9 units.

Table 7.1 Condensed, uncondensed, phenolic and etherified guaiacyl units.

	Uncondensed	Condensed
Phenolic		
Etherified		

Currently, there are few methods for determination of the degree of condensation in the phenolic and etherified C9 units in *in situ* lignin. Oxidative degradation is one such

method (*3*) which determines the degree of condensation in phenolic C9 units of *in situ* lignin. Preceding the oxidative degradation with alkaline hydrolysis of etherified linkages, it is possible to determine the degree of condensation in etherified C9 units (*4,5*). A combination of nucleus exchange[1] and nitrobenzene oxidation methods has been used to estimate the levels of uncondensed and diphenylmethane-type condensed guaiacyl units in whole *in situ* lignin (*6*).

The degree of condensation in phenolic and etherified C9 units of wood lignin has been determined by combining the periodate oxidation method with nitrobenzene oxidation or nucleus exchange (*7*). This study indicated that phenolic C9 units in lignin are appreciably less condensed than the etherified units. The method cannot be applied to lignins which have significant levels of *p*-hydroxyphenyl units, e.g. compression wood lignin (*8*). Periodate only oxidises phenolic units bearing an *ortho* methoxyl group (such as the guaiacyl nucleus), therefore free phenolic groups existing as *p*-hydroxyphenyl units will not be oxidised.

Lapierre *et al*. (*9,10*) described a method for determining the frequency of phenolic groups (and etherified units by difference) in lignin C9 units linked via β-ether linkages. Their method involved methylation of wood samples with diazomethane prior to thioacidolysis. They found that 90% of the releasable *p*-hydroxyphenyl β-ether units in *in situ* compression wood lignin were phenolic.

Smit *et al*. (*11,12*) described a method for quantification of condensed and uncondensed guaiacyl C9 units in *in situ* lignin. This involved thioacidolysis of wood to convert many of the ether inter-unit linkages to phenolic C9 units, followed by quantitative ^{31}P NMR analysis of the total thioacidolysis product. The degree of condensation in both etherified and phenolic C9 units could then be determined. This method accounts for approximately 75% of the total C9 units in lignin. Methylation of the free phenolic groups in *in situ* lignin prior to thioacidolysis/^{31}P NMR spectroscopy allows separate determination of the degree of condensation in etherified and phenolic C9 units. Since all phenolic C9 units in lignin are methylated, all peaks in the phenolic region of the ^{31}P NMR spectra will be associated with etherified C9 units originally present in wood (Figure 7.1). Using the difference between these results and those for

[1] A lignin degradation technique used to determine uncondensed units in lignin. The lignin aromatic nucleus is exchanged for phenol in the presence of boron trifluoride and excess phenol.

thioacidolysis/^{31}P NMR spectroscopy of unmethylated wood, the number of condensed phenolic C9 units in *in situ* lignin can be calculated.

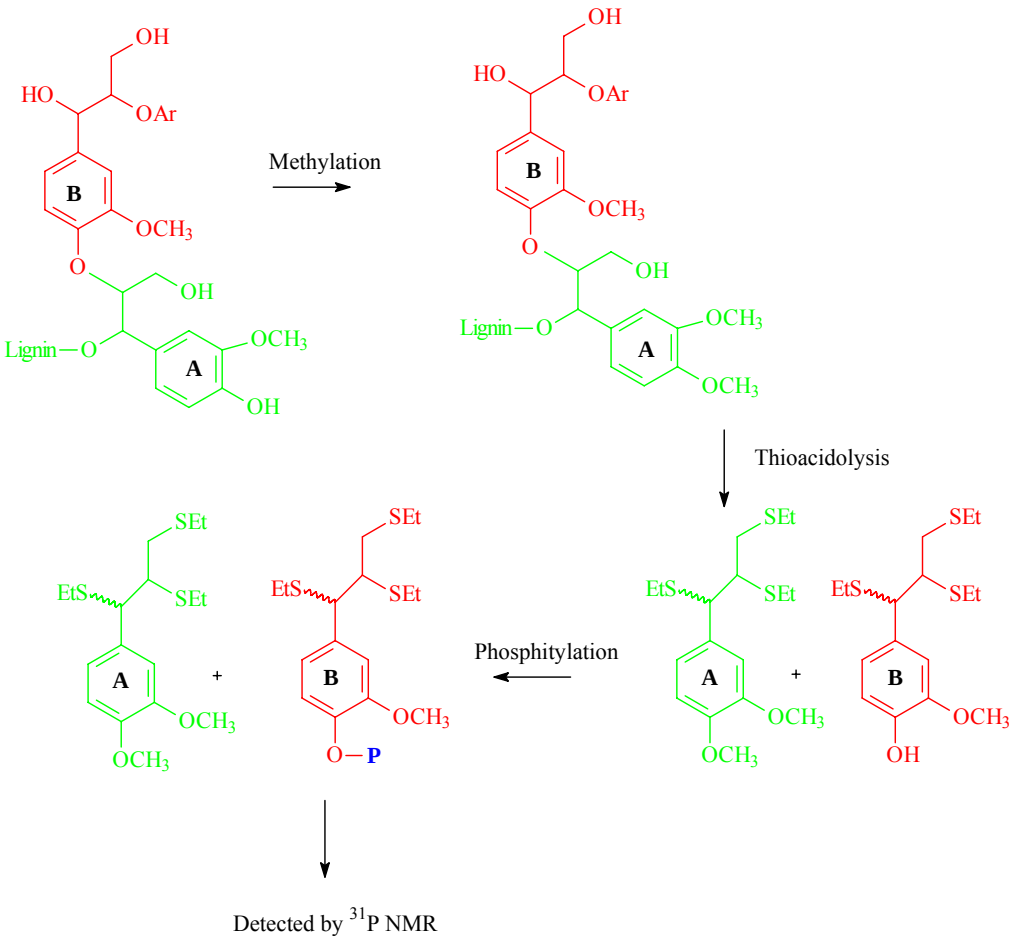


Figure 7.1 Schematic diagram showing methylation, thioacidolysis/^{31}P NMR spectroscopy analysis of phenolic (A) and etherified (B) C9 units (P = 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane).

The purpose of the present investigation was to determine the degree of condensation of phenolic and etherified C9 units in *in situ* lignin of *Pinus radiata* by a new method based on methylation/thioacidolysis/^{31}P quantitative NMR spectroscopy. Compression wood and normal wood lignin were both investigated. The results were compared with those for milled wood lignins (MWL) isolated from identical wood samples.

7.2 Materials and methods

Compression wood and opposite wood (sampled from the area of the stem directly opposite to the compression wood) were obtained from a stem disc cut at a level 5 m

above the base of a *P. radiata* tree. This was just above the point at which the terminal leader had died and an adjacent branch had subsequently grown to become the new leader. The disc had 20 growth rings.

The two wood samples were air-dried, ground in a Wiley mill to pass a 20-mesh screen, extracted with dichloromethane overnight in a Soxhlet extractor and reground to pass a 60-mesh screen. Klason and acid-soluble lignin content were determined according to Tappi Standards T222 om-88 and UM 250 respectively. The compression wood and opposite wood samples studied had Klason lignin contents of 37 and 27% respectively.

7.2.1 Milled wood lignin

Compression wood and opposite wood milled wood lignins (MWL) were prepared following the procedure described by Bjorkman (*13*), with the exception that the wood was milled in a porcelain ball mill equipped with porcelain balls under an atmosphere of N_2. Compression wood and opposite wood milled wood lignins had empirical formulae of $C_9\ H_{8.7}\ O_{3.0}\ (OCH_3)_{0.77}$ and $C_9\ H_{9.0}\ O_{3.0}\ (OCH_3)_{0.89}$ (Analysed at the Campbell Micro Analytical Laboratory, University of Otago, Dunedin) and carbohydrate contents of 1.2% and 1.3% respectively.

7.2.2 Methylation of wood

Following the method of Gierer and Norén (*14*), compression wood and opposite wood samples (200 mg) were swollen in dry dioxane (20 mL) for one week and then exhaustively methylated with an ethereal solution of diazomethane. Diazomethane solution prepared from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide was added repeatedly to the wood samples during one week period. Persistence of the yellow colour of diazomethane for at least 24 hours was taken as an indication that methylation was complete (*3*,*15*).

7.2.3 Releasable β-O-4 units

Releasable β-O-4 units were determined by the method described in Chapter 3.

7.2.4 Thioacidolysis/^{31}P NMR spectroscopy

Thioacidolysis/^{31}P NMR spectroscopy was performed by the method described in Chapter 3.

7.3 Results and discussion

7.3.1 Etherified and phenolic C9 units

Table 7.2 shows results from thioacidolysis/^{31}P NMR spectroscopic analysis of non-methylated wood, for which phenolic units (A) and etherified units (B) were both determined, and methylated wood, in which only etherified units (B) were determined (Figure 7.1). The amount of phenolic units (A) were calculated by difference.

The phenolic and etherified components in *in situ* wood lignin showed a remarkable difference in the degree of condensation (Table 7.2). In both opposite wood and compression wood only 1-5% of the phenolic units were condensed, whereas ~40% of the etherified units were condensed. Chen *et al*. (*16*) reported that in spruce wood the phenolic component of lignin measured by periodate oxidation/nitrobenzene oxidation was appreciably less condensed than its etherified counterpart. Their results indicated that the yield of nitrobenzene oxidation products from phenolic units was 2.8 times higher than that from etherified units. By means of extended permanganate oxidation of spruce wood lignin it was found that for guaiacyl C9 units the etherified C9 units were 42% condensed whereas the phenolic C9 units were 28% condensed (*5*). The results of the present study suggest a much greater difference between the phenolic and etherified units in terms of the degree of condensation than has been suggested by previous reports.

Complete methylation of phenolic C9 units is crucial for this method, as incomplete methylation might lead to erroneous results. While it is difficult to ensure methylation of the *in situ* lignin was complete, the following indirect evidence can be offered:

a. A well-established methylation procedure was followed (*9,10,14,15,17*).
b. The free phenolic content of *P. radiata* opposite wood (14%) (Table 7.2) is consistent with values determined by periodate oxidation and UV methods for other softwoods (*7,18*)

c. The percentage of condensed guaiacyl units obtained by permanganate oxidation of diazomethane-methylated MWL (*19*) and MWL methylated with dimethyl sulphate (*20*) compare favourably with results obtained here (~35%), showing that diazomethane can methylate condensed units as effectively as other methylating agents (*3*).

Table 7.2 Thioacidolysis/^{31} P NMR spectroscopy results for *in situ* lignin and MWL.

	C9 units - mmol/g lignin				OH/ 100 C9[1]	% Cond. G
	Uncond. H	Uncond. G	Cond. G	Total		
OPPOSITE WOOD						
Lignin *in situ*						
Total units (A+B)[2]	0.06	2.63	1.35	4.04		34±4[4]
Etherified units (B)	Trace	1.96	1.34	3.30		41±4
Phenolic units (A)[3]	0.06	0.67	0.01	0.74	14	1±4
MWL						
Total units	0.12	2.48	1.38	3.98		36±4
Etherified units[3]	0.02	1.80	0.96	2.78		35±4
Phenolic units	0.10	0.68	0.42	1.20	22	38±4
COMPRESSION WOOD						
Lignin *in situ*						
Total units	0.45	2.34	1.48	4.27		39±4
Etherified units	Trace	1.65	1.44	3.09		47±4
Phenolic units	0.45	0.69	0.04	1.18	22	5±4
MWL						
Total units	0.41	2.34	1.31	4.06		36±4
Etherified units	0.07	1.67	0.97	2.71		37±4
Phenolic units	0.34	0.67	0.34	1.35	25	34±4

[1] Assume a molecular weight of 183 g/mol for a C9 unit in lignin (*21*), which means there is a total of 5.34 mmol C9 units/g lignin. Therefore, 0.74 mmol/g lignin equals 14 OH/100 C9 units (0.74/5.34 = 0.135).
[2] For A and B refer to Figure 7.1.
[3] Calculated by difference.
[4] % error.

The proportion of phenolic C9 units in compression wood (22%) was consistently higher than in opposite wood has been reported previously (*22*,*23*). This can be attributed to elevated levels of *p*-hydroxyphenyl units in compression wood (Chapters 3 and 4), which mainly exist as phenolic end groups (*9*).

7.3.2 Etherified and phenolic C9 units in isolated lignin

Milled wood lignin is often used for studies of lignin structure. It is interesting to compare the degree of condensation of etherified and phenolic C9 units in MWL with that of the corresponding *in situ* lignin. Isolated milled wood lignin samples were analysed directly by ^{31}P NMR spectroscopy and then by thioacidolysis/^{31}P NMR spectroscopy to determine the degree of condensation of phenolic C9 units and the sum of phenolic and etherified units respectively (*12*). The etherified units were then calculated by difference.

The results in Table 7.2 show that 37% of the phenolic units in MWL were condensed in contrast only 5% condensed in *in situ* lignin. Similar results have been reported from quantitative ^{31}P NMR spectroscopy (*12*,*24*) and by permanganate oxidation methods (*19*,*20*). Isolated lignins had a considerably higher free phenolic content, 22-25% (Table 7.2) than *in situ* lignin. This might have been due to cleavage of etherified β-O-4 inter-unit linkages caused by ball milling, which would explain the elevated degree of condensation of the phenolic C9 units (Table 7.2) of MWL when compared with *in situ* lignin. Values of 18-33% free phenolic units has been reported in the literature for normal wood MWLs (*18*). One factor contributing to the variability in reported free phenolic contents might be a lack of uniformity in the efficiency of the milling process used in the MWL preparation (*2*).

Milled wood lignin originating from opposite wood had more phenolic C9 units (22% *vs.* 14%) but fewer releasable β-O-4 units (1041 *vs.* 1589 μmol/g lignin) than the actual opposite wood. The findings here show that caution is required when using MWL to provide information on the phenolic unit content of *in situ* wood lignins.

7.3.3 Comparison of phenolic and etherified releasable β-ethers in in situ wood lignin

The levels of phenolic and etherified releasable β-ethers of the same wood samples used for the earlier study, were also determined following the method described by Lapierre *et al*. (*9*) (Figure 7.2).

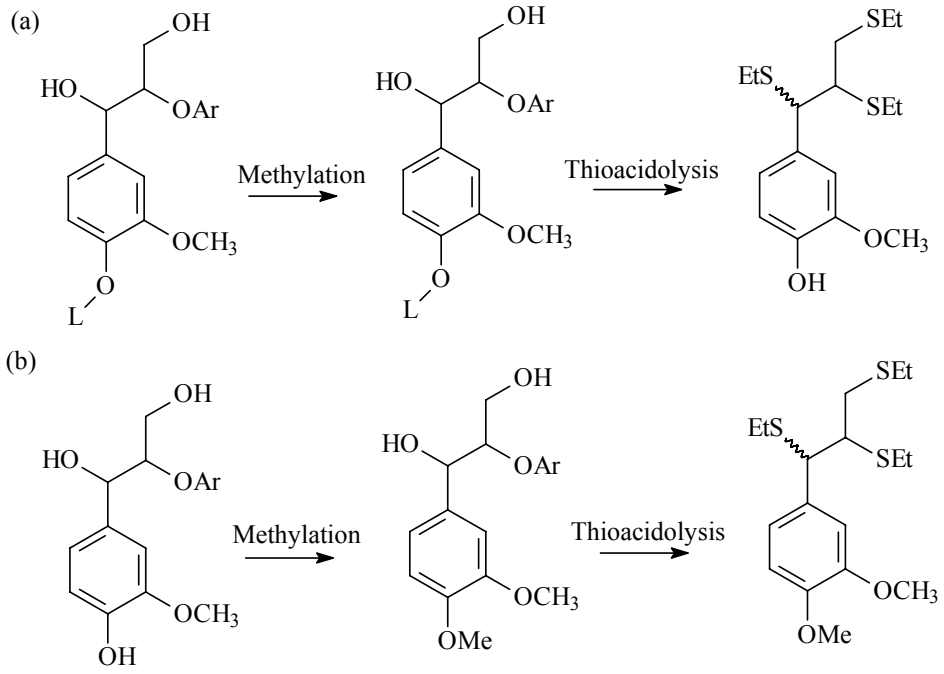


Figure 7.2 Thioacidolysis of original wood lignin and diazomethane-methylated lignins showing main recovered C9 units from β-O-4 linkages in a) non terminal C9 units b) terminal C9 units. L = aliphatic C in lignin.

For both opposite wood and compression wood, essentially all the *p*-hydroxyphenyl β-O-4 units were phenolic, whereas only approximately one quarter of the guaiacyl units are phenolic (Table 7.3). The presence of *p*-hydroxyphenyl β-ether units principally in the form of free phenolic units has been reported previously (*10*). This is consistent with the thioacidolysis/^{31}P NMR spectroscopy results, which showed that the uncondensed *p*-hydroxyphenyl C9 units were largely phenolic (Table 7.2). Although, this has already been reported for *P. pinaster* compression wood (*10*) it is interesting to find that *p*-hydroxyphenyl units exist as free phenolic forms irrespective of the type of wood i.e. compression wood or opposite wood.

Table 7.3 Phenolic and etherified releasable β-O-4 units.

		μmol/g lignin			
		Opposite wood		Compression Wood	
		Original	Methylated	Original	Methylated
Preswelled					
H	Etherified	12	Trace	235	6
	Phenolic		23		140
G	Etherified	1577	844	1312	686
	Phenolic		277		270
H+G	Total β-ethers	1589	1144	1547	1102
Unswelled					
H	Etherified	12	Trace	154	Trace
	Phenolic		24		147
G	Etherified	1098	986	834	684
	Phenolic		283		258
H+G	Total β-ethers	1110	1293	988	1089

7.3.4 Effect of preswelling of wood prior to thioacidolysis

Table 7.3 shows that total releasable β-ethers measured by thioacidolysis following methylation were only ~70% of those measured by thioacidolysis of non-methylated compression wood and opposite wood. This appears to be due to the fact that all wood samples described in this thesis were preswelled in water overnight prior to thioacidolysis, as described by Önnerud and Gellerstedt (*23*). The second part of Table 7.3 shows the results of thioacidolysis carried out on air-dried samples. By comparison of the two sets of results it can be concluded that:

1. Present data confirm previous findings i.e. in unmodified wood, preswelling increases the yield of releasable β-ethers (here up to 40%).
2. The increase in yield was observed for both H and G releasable β-ethers
3. Preswelling had no significant effect on methylated samples.

Önnerud and Gellerstedt (*23*) suggested that preswelling improves accessibility of thioacidolysis reagents to the lignin, leading to more complete degradation. It is not surprising that methylation changes the extent to which wood swells, and therefore the

level of releasable β-ethers measured, improved accessibility of thioacidolysis reagents cannot explain all the effects of preswelling. If preswelling leads to increased removal of lignin from wood, an increase in proportion of C9 units analysed by thioacidolysis/^{31}P NMR spectroscopy might also be expected. Table 7.4 presents the results of thioacidolysis/^{31}P NMR spectroscopic analysis of two wood samples before and after swelling. In both wood samples the proportion of C9 units analysed in swelled and unswelled wood was similar. Levels of uncondensed and condensed units and the proportion of condensed units, i.e. β-5, 5-5 and 4-O-5, were also similar in both cases. From these results, it can be concluded that preswelling does not lead to increased proportions of C9 units being analysed.

Table 7.4 Yields for thioacidolysis/^{31}P NMR spectroscopy with and without preswelling of wood samples.

	C9 units (mmol/g lignin)				% C9 Units[1]	% Cond. G
	Uncond. H	Uncond. G	Cond. G	Total		
OW						
Unswelled	0.06	2.59	1.38	4.03	75	35
Preswelled	0.06	2.56	1.36	3.98	75	35
CW						
Unswelled	0.41	2.35	1.44	4.20	79	34
Preswelled	0.49	2.33	1.51	4.33	80	35

[1]Assume a molecular weight of 183 g/mol for a C9 unit in lignin (*21*).

One explanation for the above results is that traces of water or alcohol in the wood following preswelling react partially with BF_3 to form more reactive Brønsted acids such as HBF_4. These modify degradation of lignin during thioacidolysis to produce higher levels of releasable β-ethers without changing the proportion of C9 units released.

During thioacidolysis of β-ethers, formation of dithioacetal occurs as a competitive side reaction to the generation of trithioethers (*25*) (Figure 7.3). If the extent of dithioacetal formation was repressed the yields of the main trithioether products would be increased. For one sample, levels of the dithioacetal produced from thioacidolysis of preswelled sample were <10% of those from thioacidolysis of the air-dried samples.

This suggests that preswelling may increase the efficiency with which β-ethers are converted to trithioethers by inhibiting dithioacetal formation. Unfortunately, it was not possible to quantify levels of dithioacetal produced as GC-MS quantification was performed in selected ion monitoring mode scanning for main thioacidolysis products. Further work is clearly required to investigate these suggestions further.

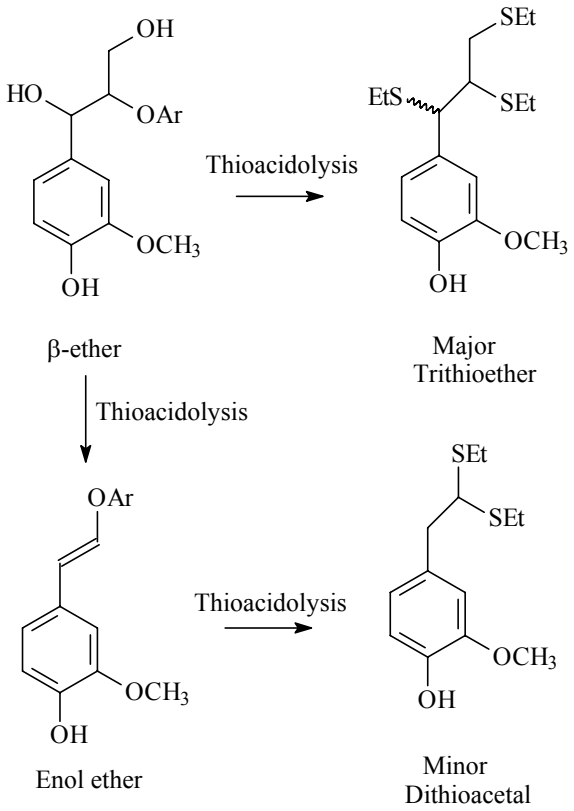


Figure 7.3 Formation of dithioacetal product from a side reaction of β-ethers.

7.4 Conclusions

Thioacidolysis of methylated *in situ* wood lignin followed by quantitative ^{31}P NMR spectroscopy is a useful method for determining the degree of lignin condensation of phenolic and etherified C9 units in *in situ* lignins. By this method it was found that phenolic C9 units in *in situ* lignin were considerably less condensed than etherified C9 units in both compression wood and normal wood.

The phenolic component of isolated lignin was more condensed than that of *in situ* lignin. These findings indicate that caution is required when using milled wood lignin to provide information on phenolic units in *in situ* wood lignins.

Ninety five percent of the releasable H β-O-4 units and 23% of G releasable β-O-4 units occurred as phenolics in both compression wood and opposite wood.

7.5 References

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CHAPTER 8

8 Summary and Conclusions

The ultimate goal of this study was to develop a quantitative chemical test to measure the degree of compression wood development in *Pinus radiata* wood samples. The intention was to develop the test for *P. radiata* since it is the important commercial species in New Zealand. However, it would be applicable to other softwood species, since the chemistry of compression wood is similar in all softwoods.

The main objective of the research was to ascertain how the chemical composition, structure and amounts of lignin, as well as the amounts and ratios of specific sugars, changes with compression wood severity. Results showed that, increasing severity of compression wood is associated with:

- increase in lignin and galactose levels and decrease in glucose and mannose levels
- increase in releasable *p*-hydroxyphenyl β-ether levels
- decrease in releasable guaiacyl β-ether levels
- increase in uncondensed *p*-hydroxyphenyl C9 units and decrease in uncondensed guaiacyl C9 units

There was a good agreement between compression wood severity assessed by fluorescence microscopy and the measured lignin content of above 31%. A number of chemical parameters changed linearly with compression wood severity, so could potentially be used as indicators of compression wood severity. These were the amount of lignin, galactose, galactose/glucose and *p*-hydroxyphenyl content in lignin. Parameters based on the *p*-hydroxyphenyl unit content in lignin appears to offer the greatest potential as a chemical indicator of compression wood severity since they spanned a larger range relative to the normal wood levels and were not influenced by the morphological origin of the wood samples.

The results showed that the chemical differences between compression wood and normal wood were independent of the morphological origin of the wood sample.

A secondary objective of the study was to isolate galactan found in the compression wood of *P. radiata* since this has not been investigated before. It was found that galactan isolated from compression wood of *P. radiata* was largely composed of (1→4)-linked β-D-galactopyranose residues and had no evidence to indicate the presence of any branches.

A new method based on thioacidolysis and ^{31}P quantitative NMR spectroscopy for estimation of the degree of lignin condensation of the phenolic and etherified C9 units in *in situ* wood lignin was developed. Using this method it was found that phenolic C9 units in *in situ P. radiata* lignin were considerably less condensed than etherified C9 units in both compression wood and normal wood. As the method depends heavily on the completeness of the methylation of the *in situ* lignin further verification of the completeness of the methylation would be desirable.

A difficulty faced during the course of this research was the lack of a quantitative measure of compression wood severity to compare with the chemistry. A semi-quantitative assessment of compression wood severity was carried out using the fluorescence microscopy. A shortcoming of this approach was that an exact match between the fluorescence microscopy characterisation and the chemical analysis could not be achieved as chemical analysis requires a much larger sample (2×2 cm^2) than the area assessed by the microscopy. Our approach was to determine the compression wood severity semi-quantitatively by fluorescence microscopy at number of areas in the sample and then to assume that this represent the severity of the whole sample used for the chemical analysis.

A unique feature of this study is the characterisation of the severity of compression wood samples using the fluorescence microscopy, and relating chemical composition with the anatomical features of compression wood. Chemical composition of mild compression wood is reported here for the first time.

Future research

Overall, the main finding of this study is the importance of *p*-hydroxyphenyl units in *in situ* lignin as a measure of compression wood severity. There are few potential areas of further study arising out of the research.

- One is the validation of the relationship between the H β-ether units or the H/G β-ether lignin ratio with practically important wood properties like shrinkage.

- Another issue is the need for faster instrumental or chemical methods to establish the H lignin units in a wood sample.

- A reasonable agreement between lignin content and compression wood severity assessed by fluorescence microscopy was observed in this study. Since lignin content varies with age and position within the tree further verification of the relationship between lignin content and the compression wood severity assessed by microscopy is needed.

- The number of samples in this study had to be limited to 27 due to the time consuming nature of wet chemistry methods. This prevented us from exploring the potential of NIR and IR for detection of H units. If a large data set was available NIR and IR techniques in conjunction with multivariate analysis could be explored as a rapid means of detecting H/G β-ether ratio of lignin.

- The use of pyrolysis as a rapid method of determining H/G ratio of wood lignin was not successful in our case. Pyrolysis may be useful as a rapid method for detecting H/G ratio if tested using large data sets combined with multivariate analysis.

- The effect of age on the compression wood development could not be answered conclusively during the course of this thesis and needs to be addressed in the future.

Appendix A

Statistical Analysis

Repeatability

To evaluate repeatability a sample of dried extracted ground wood were subjected to repeated analyses. Least significance difference (LSD) values calculated are valid for comparing two dried ground extracted wood samples each analysed in duplicate. Confidence limits were constructed using Student's t distribution. This was possible because of the small population size and the assumption that the measured values were random with an approximate normal distribution. The general equation for the construction of a (1-α) 100% confidence interval, where n<30, is presented below:

$$\bar{x} - t_{\alpha/2}\frac{s}{\sqrt{n}} \prec \mu \prec \bar{x} + t_{\alpha/2}\frac{s}{\sqrt{n}}$$

Where $t_{\alpha/2}$ is the Student's t value with n-1 degrees of freedom, above which the area under the Student's t distribution curve equals α/2. S = standard deviation and n = number of samples. The percent error and LSD's (LSD = S × $t_{\alpha/2}$) associated with 95% confidence intervals (*p*) are presented in Tables A.2, A.4 and A.6.

Table A.1 Summative analysis data used for calculation of LSD.

g/100 g oven dry extractive-free wood					
Klason lignin	Arabinose	Galactose	Glucose	Xylose	Mannose
27.1	1.3	1.6	44.5	5.3	11.3
26.8	1.3	1.6	43.2	5.5	11.9
26.8	1.3	1.5	41.3	5.6	12.4
27.0	1.4	1.7	44.5	6.2	13.5
27.2	1.4	1.6	43.1	5.8	12.7
26.1	1.3	1.4	40.3	5.4	11.9
27.2	1.2	1.4	42.6	5.4	12.1
28.3	1.3	1.6	45.0	6.2	13.3
27.4	1.4	1.8	44.3	4.9	12.3
27.4	1.4	1.7	45.0	5.0	11.9
27.5	1.4	1.8	44.9	4.9	12.4
27.3	1.4	1.8	45.6	5.1	11.7
27.7	1.1	1.5	42.9	6.2	12.1
26.5	1.3	1.6	42.1	5.7	12.7
27.9	1.3	1.5	45.1	5.1	11.7
27.3	1.3	1.4	43.2	4.7	11.0
27.3	1.3	1.5	42.6	5.4	12.1
27.5	1.3	1.5	42.5	5.4	12.1

Table A.2 Repeatability for summative analysis.

	Mean	SD	Half 95 % Confidence interval (for mean of 18)	% error (for mean of 18)	LSD for comparing means of duplicates (5% level)
Lignin	27.2	0.507	0.2522	0.9	1.07
Arabinose	1.31	0.070	0.0345	2.6	0.15
Galactose	1.58	0.128	0.0638	4.0	0.27
Glucose	43.48	1.467	0.7295	1.7	3.10
Xylose	5.42	0.454	0.2257	4.2	0.96
Mannose	12.17	0.615	0.3057	2.5	1.30

Table A.3 Thioacidolysis/^{31}P NMR spectroscopy data used for calculation of LSD.

C9 units (mmol/g lignin)						
β-5	4-O-5	5-5	Condensed G	Uncondensed G	Uncondensed H	Total
0.32	0.28	0.66	1.26	2.51	0.09	3.86
0.36	0.25	0.71	1.31	2.50	0.08	3.89
0.34	0.28	0.76	1.37	2.61	0.07	4.05
0.40	0.22	0.74	1.41	2.52	0.09	4.01
0.34	0.27	0.67	1.46	2.45	0.09	4.00
0.39	0.30	0.65	1.36	2.60	0.08	4.04

Table A.4 Repeatability for thioacidolysis/^{31}P NMR spectroscopy.

Lignin functional group	Mean	SD	Half 95 % Confidence interval (mean of 6)	% error (mean of 6)	LSD for comparing means of duplicates (5% level)
β-5	0.36	0.031	0.032	9.0	0.08
4-O-5	0.27	0.027	0.028	10.5	0.07
5-5	0.70	0.046	0.048	6.8	0.12
condensed G	1.36	0.069	0.073	5.3	0.18
Uncondensed G	2.53	0.062	0.065	2.7	0.16
Uncondensed H	0.06	0.010	0.010	10.7	0.02

Table A.5 Thioacidolysis data used for calculation of LSD.

μmol/g lignin		
G+H	G	H
1226	1216	10
1063	1048	15
1110	1094	16
1092	1080	12
1202	1188	14
958	943	15
991	975	16
931	921	10
935	924	11
1027	1016	11
938	924	14
922	906	16
1211	1199	12
1012	997	15
1159	1139	20
1189	1173	16

Table A.6 Repeatability for thioacidolysis.

	Mean	SD	Half 95 % Confidence interval (mean of 16)	% error (mean of 16)	LSD for comparing means of duplicates (5% level)
G+H	1060	111	59.26	6	237
H	14	3	1.46	10	6
G	1046	111	59.19	6	237

Appendix B

Analytical Data

Table B.1 Summative composition and releasable β-O-4 units.

	Summative component (g/100 g oven dry extractive-free wood)						Releasable β-O-4 units (μmol/g lignin)	
Sample	Lignin	Ara	Gal	Glc	Xyl	Man	G	H
Ring 3-4								
Opposite	27.9	1.9	4.5	41.1	6.7	9.0	1,655	11
Mild	32.9	1.6	8.0	35.0	5.9	7.2	1,616	105
Severe	38.6	1.4	14.0	28.2	5.0	5.0	1,361	287
Ring 10 Opposite	27.3	1.7	2.4	45.1	6.5	10.6	1,848	9
Mild	31.4	1.6	4.7	43.3	5.3	10.9	1,543	40
Severe	35.4	1.3	10.1	35.0	5.1	6.7	1,636	193
Ring 15-16								
Opposite	27.1	1.3	1.8	46.6	4.6	11.7	1,339	14
Mild	31.3	1.2	4.5	39.7	4.6	9.0	1,453	90
Severe	35.6	0.9	8.2	33.8	4.6	6.9	1,144	235
Branch								
OW	30.7	1.6	2.9	37.5	6.8	9.3	1,420	28
CW	39.6	1.1	12.2	25.4	4.2	5.8	1,057	393
Knot OW	30.1	1.7	2.7	35.7	6.3	9.4	1526	40
CW	38.0	1.0	10.2	27.0	3.7	7.2	1285	313
Stem (above and below branch)								
OW	29.9	1.6	2.4	40.4	5.8	11.0	1305	18
CW	39.1	1.3	10.0	28.9	3.4	6.9	1107	253
Young wood - 1 year								
OW	30.0	2.0	2.8	40.0	7.7	8.9	1707	Trace
CW	31.6	2.0	6.7	35.8	6.7	7.9	1548	61
Young wood - 2 year								
OW	27.4	2.2	2.0	43.2	8.2	10.6	1,342	11
CW	34.9	1.6	9.0	33.7	5.3	8.2	1,401	127

Table B.2 Thioacidolysis/ ^{31}P NMR spectroscopy results.

Sample	C9 units (mmol/g lignin)							
	β-5	4-O-5	5-5	Cond. G	Uncond. G	Uncond. H	Total C9	% Cond. G
Ring 3-4								
Opposite	0.32	0.28	0.66	1.26	2.51	0.08	3.86	33
Mild	0.38	0.20	0.74	1.30	2.33	0.26	3.89	36
Severe	0.38	0.29	0.67	1.34	2.08	0.56	3.99	39
Ring 10								
Opposite	0.40	0.25	0.67	1.32	2.66	0.07	4.05	33
Mild	0.45	0.25	0.61	1.31	2.51	0.11	3.93	34
Severe	0.42	0.26	0.70	1.38	2.40	0.41	4.19	37
Ring 15-16								
Opposite	0.36	0.25	0.71	1.31	2.50	0.05	3.87	34
Mild	0.38	0.20	0.75	1.32	2.31	0.19	3.82	36
Severe	0.34	0.23	0.84	1.40	2.20	0.37	3.97	39
Branch								
OW	0.34	0.28	0.76	1.37	2.61	0.08	4.07	34
CW	0.42	0.28	0.71	1.41	2.05	0.74	4.20	41
Knot								
OW	0.33	0.33	0.67	1.33	2.46	0.09	3.88	34
CW	0.20	0.20	0.36	1.36	2.18	0.56	4.10	33
Stem (above and below branch)								
OW	0.34	0.27	0.85	1.46	2.27	0.09	3.82	39
CW	0.27	0.30	0.48	1.52	2.19	0.5	4.21	41
Young wood - 1 year								
OW	0.36	0.27	0.62	1.25	2.72	0.07	4.03	31
CW	0.43	0.29	0.76	1.48	2.57	0.15	4.20	37
Young wood - 2 year								
OW	0.44	0.22	0.74	1.41	2.52	0.07	4.00	36
CW	0.40	0.28	0.74	1.42	2.35	0.31	4.09	38

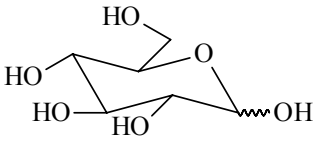
Table B.3 Releasable β-ethers and uncondensed units in lignin.

			Releasable β-ethers (mmol/g)		Uncondensed C9 units (mmol/g)		Releasable β-ethers/ Uncondensed C9 units	
Sample		Lignin	G	H	G	H	G ratio	H ratio
Ring 3-4								
	Opposite	27.9	1.655	0.011	2.51	0.08	0.66	0.14
	Mild	32.9	1.616	0.105	2.33	0.26	0.69	0.40
	Severe	38.6	1.361	0.287	2.08	0.56	0.65	0.51
Ring 10								
	Opposite	27.3	1.848	0.009	2.66	0.07	0.69	0.13
	Mild	31.4	1.543	0.040	2.51	0.11	0.61	0.36
	Severe	35.4	1.636	0.193	2.40	0.41	0.68	0.47
Ring 15-16								
	Opposite	27.1	1.339	0.014	2.50	0.05	0.54	0.28
	Mild	31.3	1.453	0.090	2.31	0.19	0.63	0.47
	Severe	35.6	1.144	0.235	2.20	0.37	0.52	0.64
Branch								
	OW	30.7	1.420	0.028	2.61	0.08	0.54	0.35
	CW	39.6	1.057	0.393	2.05	0.74	0.52	0.53
Knot								
	OW	30.1	1.526	0.040	2.46	0.09	0.62	0.44
	CW	38.0	1.285	0.313	2.18	0.56	0.59	0.56
Stem (above and below branch)								
	OW	29.9	1.305	0.018	2.27	0.09	0.57	0.20
	CW	39.1	1.107	0.253	2.19	0.5	0.51	0.51
Young wood - 1 year								
	OW	30.0	1.707	-	2.72	0.07	0.63	-
	CW	31.6	1.548	0.061	2.57	0.15	0.60	0.41
Young wood - 2 year								
	OW	27.4	1.342	0.011	2.52	0.07	0.53	0.16
	CW	34.9	1.401	0.127	2.35	0.31	0.60	0.41

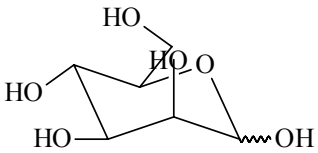
Appendix C

Chemical structures of monosaccharides

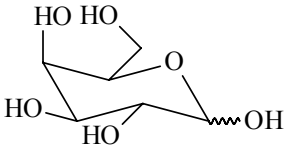
Hexoses



D-glucopyranose

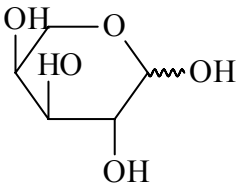


D-mannopyranose

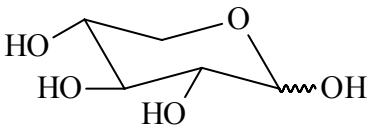


D-galactopyranose

Pentoses

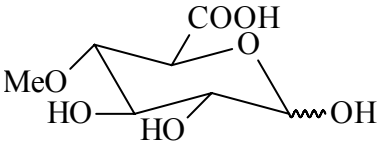


L-arabinofuranose

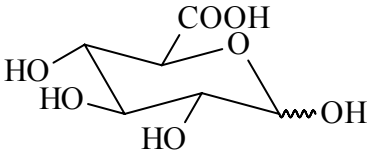


D-xylopyranose

Uronic acids



4-*O*-methyl-D-glucuronic acid



D-galacturonic acid