– 9 –

Lignins: Major Sources, Structure and Properties

Göran Gellerstedt and Gunnar Henriksson

ABSTRACT

Lignin is one of the most predominant biopolymers present in plants. Together with cellulose and hemicelluloses, lignin builds up the cell wall in an arrangement which is regulated on the nano-scale and results in lignin–carbohydrate network structures. The molecular complexity of lignin renders all isolation and identification processes difficult and, consequently, many structural questions still remain. In this chapter, our present knowledge about the formation of lignin in plants, its presence in different types of plants as well as several different approaches taken to reveal the chemical structure, is summarized. Furthermore, a brief discussion about the chemical changes introduced in lignin as the result of different types of delignification processes, such as kraft and sulphite pulping and steam explosion, is included.

Keywords

Analytical methods, Annual plants, Cellular structures, Dehydropolymerizate (DHP), Hardwoods, Kraft lignins, Lignification, Lignins, Lignosulphonates, Milled wood lignins (MWL), Softwoods, Steam explosion lignins

9.1 INTRODUCTION

Plants are eukaryotic organisms with an ability to utilize light for the fixation of carbon dioxide, having cell walls rich in cellulose and with an ability to use starch as nutrition storage inside the cells. In the evolution of plants, lignin (the term lignin is derived from the Latin word for wood, *lignum*) was introduced some 440 million years ago when the group of vascular plants started to develop. Thereby, a new cell type, the tracheid, was formed typified by its elongated feature and its thick hydrophobic cell wall. In addition to cellulose and lignin, all vascular plants also contain other polysaccharides, hemicelluloses and low molecular mass compounds, extractives. The presence of tracheids permitted an efficient transport of water in the plant and provided the strength necessary for the development of larger species. Simultaneously, the development of root systems took place, thus also allowing plants to grow in dryer environments.

The first vascular plants to develop were vascular cryptogams reproduced using spores and, today, such plants can still be found as the herb families of club mosses, ferns, horsetails and in fern trees. In the further evolution of plants, seed fertilization developed some 360 million years ago and paved the way for an expansion of novel types of trees which successively overruled the vascular cryptogam trees due to a more efficient reproduction system and a greater ability to colonize dryer areas of land. The first trees to develop all had naked seeds often organized in cones and are referred to as gymnosperms. About 150 million years ago, a dramatic new development occurred with plants-carrying flowers, the fertilization being done by insects and with seeds developed inside a fruit body. Such plants, the angiosperms, rapidly took over in importance and, today, more than 90 per cent of all land-living plant species are of this type. Simultaneously, the angiosperms developed more advanced types of leafs and the

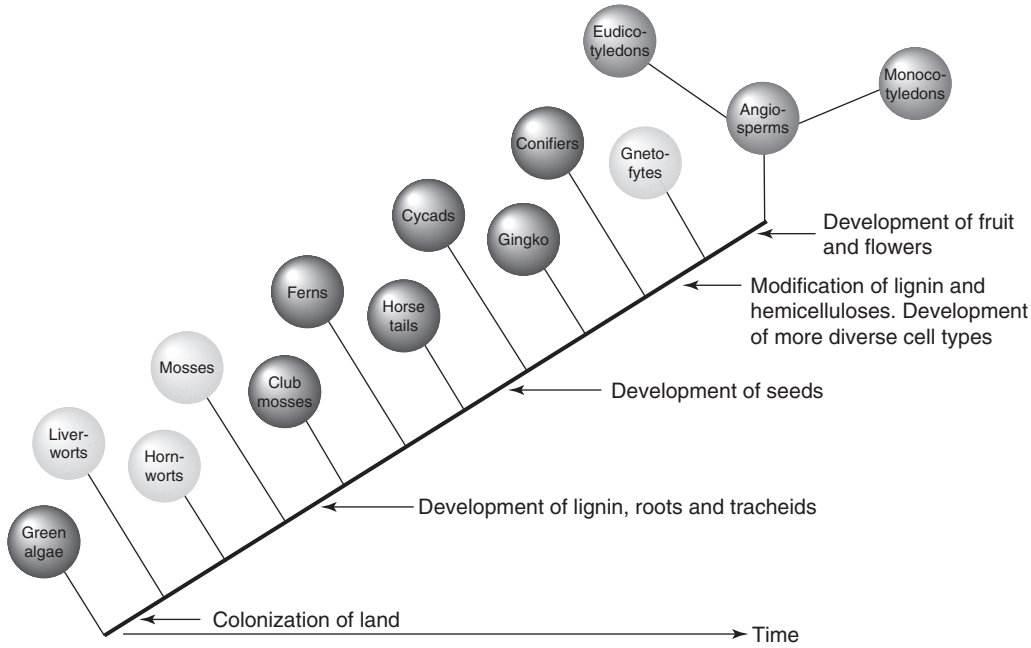

Figure 9.1 A schematic view of the evolution of plants.

cells became more specialized with vessel cells taking care of the water and nutrition transport. The structure of lignin and hemicelluloses was modified in comparison to the gymnosperms. Usually, the angiosperms are divided into two classes, *viz*. the monocotyledons, which include species such as grasses, bamboo and palm trees, and the eudicotyledons represented among others by leaf-carrying trees like birch, aspen and eucalyptus. The woody plants of the latter group are referred to as hardwoods. A simplified schematic representation of the evolution of plants is given in Fig. 9.1.

9.2 NOMENCLATURE OF LIGNIN

Lignins are formed by polymerization of cinnamyl alcohols (monolignols) which differ in structure depending on plant type. In coniferous wood, the lignin is built up almost exclusively by coniferyl alcohol (G-units) with minor amounts of coumaryl alcohol (H-units) present. The latter is, however, a major constituent in compression wood lignin. In hardwoods, on the other hand, both coniferyl alcohol and sinapyl alcohol (S-units) are used as building blocks and in monocotyledonous tissue, all three alcohols are used as lignin precursors [1–3]. Figure 9.2 shows the numbering system and the structure of the three major monolignols, together with some minor monolignols found as lignin end-groups or present in specific plants [4, 5]. Table 9.1 shows the degree of participation of the major monolignols in different types of plants.

9.3 BIOSYNTHESIS OF MONOLIGNOLS AND THE FORMATION OF LIGNIN

The three major monolignols (Fig. 9.2) used to synthesize lignin polymers are formed in the cytoplasm via the shikimate pathway which produces phenylalanine as key intermediate [7–9]. Through further enzyme mediated deamination, hydroxylation, reduction and methylation reactions, the final lignin precursors are formed as depicted in Fig. 9.3.

The further reactions of monolignols in the plant cell wall to form lignin may occur through an initial laccase or peroxidase oxidation of the monolignol giving rise to a resonance-stabilized phenoxy radical as depicted in Fig. 9.4 [10–12]. This step is followed by a coupling reaction and model experiments have shown that the first product, the

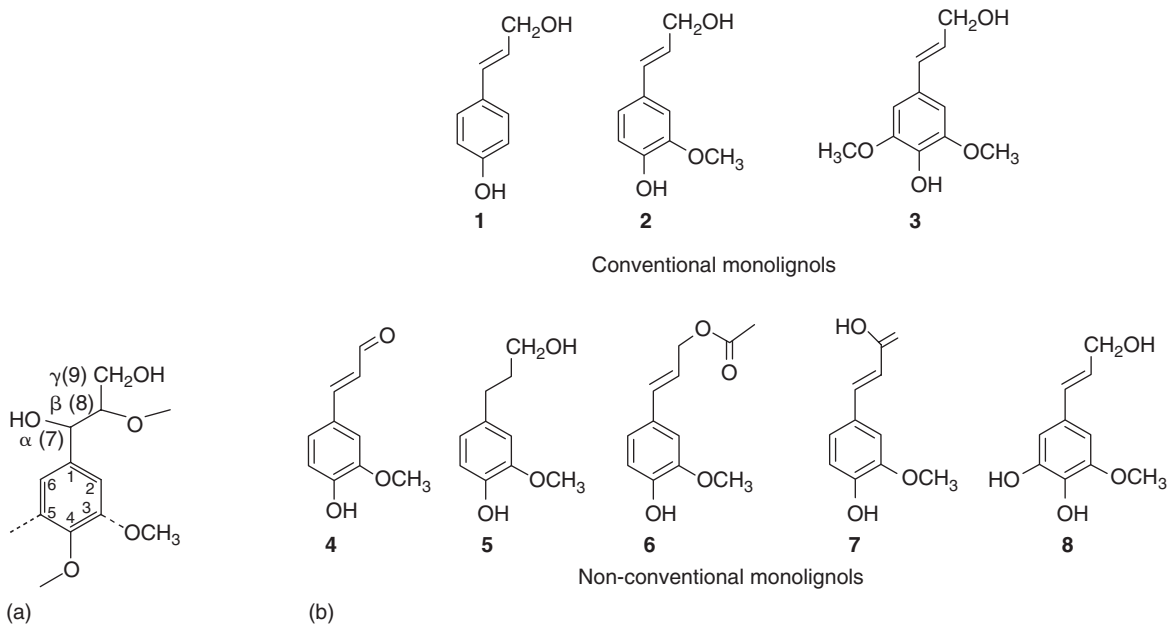

Figure 9.2 (a) Numbering system in monolignols. (b) Types of monolignols found as building blocks in lignin. 1 = *p*-coumaryl alcohol (H-unit), 2 = coniferyl alcohol (G-unit), 3 = sinapyl alcohol (S-unit), 4 = coniferaldehyde, 5 = dihydroconiferyl alcohol, 6 = coniferyl alcohol-9-acetate, 7 = ferulic acid, 8 = 5-hydroxyconiferyl alcohol.

Table 9.1

The participation of different monolignols in lignin from various plants

Plant type	*p*-Coumaryl alcohol	Coniferyl alcohol	Sinapyl alcohol
	(%)		
Coniferous; softwoods	<5[a]	>95	0[b]
Eudicotyledonous; hardwoods	0–8	25–50	45–75
Monocotyledonous; grasses	5–35	35–80	20–55

[a] Higher amount in compression wood.
[b] Some exceptions exist [6].

dimer, will be formed by involvement of the β-carbon in one unit and either the phenolic hydroxyl, the aromatic C5 (only in G- or H-units) or the β-carbon in the other [10–12]. It should be noted that the theoretically possible coupling products between two C5 centred radicals or between a phenoxy and a C5 radical are not formed to any noticeable extent. The stepwise further growth of the polymer is thought to involve an end-wise addition of a new monolignol radical to the growing polymer chain. In the latter, however, only the phenolic hydroxyl, the aromatic C5 or C1 positions are available for coupling. Thus, the overall frequency of inter-unit linkages involving the C–β carbon can be expected to be completely dominant in any lignin. Coupling in the aromatic C1 position takes place to a low extent and predominantly results in the formation of a spirodienone structure with a secondary lignin fragmentation reaction as an alternative [13]. Other possible modes of coupling may occur if two growing polymer chains are in close proximity, by formation of C5/C5 (in G-units) or phenolic hydroxyl/C5 linkages. Thereby, branching or crosslinking points in the lignin polymer are created. Branching points in the growing lignin polymer are also created by the formation of dibenzodioxocin structures. These are prevalent in guaiacyl lignins and formed by internal trapping after coupling of a C–β radical with a 5-5′ phenoxy radical [14, 15]. The various types of inter-unit linkages and their abbreviated nomenclature are depicted in Fig. 9.5.

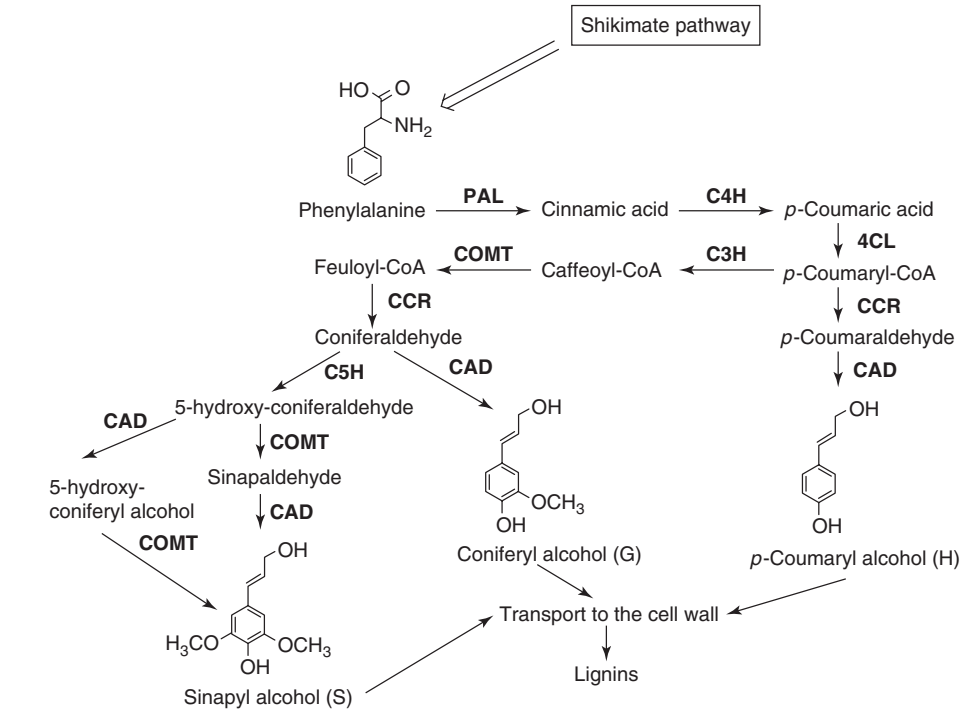

Figure 9.3 The lignin biosynthesis pathway. Abbreviations of enzymes are: PAL = phenylalanine–ammonia-lyase; C4H = cinnamate 4-hydroxylase; 4CL = hydroxycinnamate:CoA-ligase; C3H = 4-hydroxycinnamate 3-hydroxylase; COMT = S-adenosyl-methionine:caffeate/5-hydroxyferulate-O-methyltransferase; CAD = hydroxycinnamyl alcohol dehydrogenase; C5H = coniferaldehyde-5-hydroxylase.

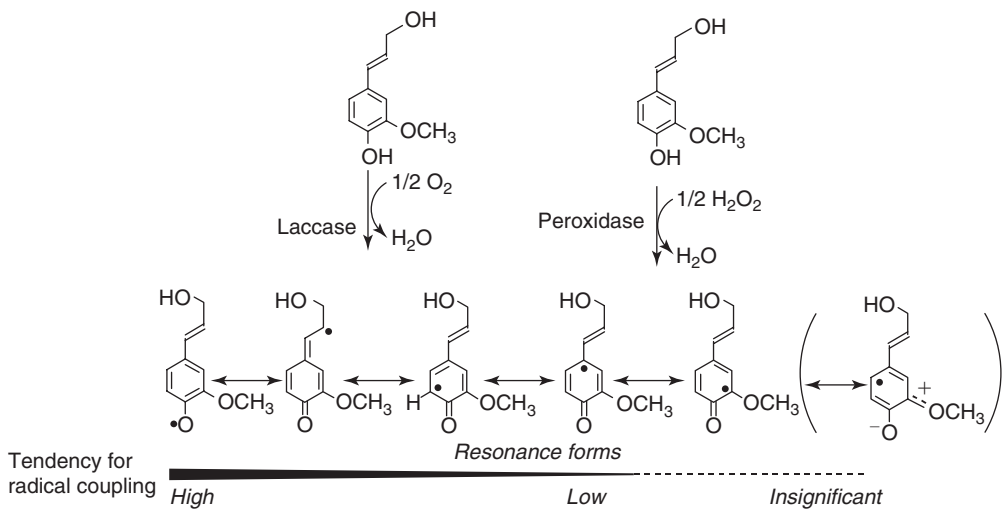

Figure 9.4 Enzymatic formation of resonance-stabilized monolignol radicals. The relative reactivity in coupling reactions is indicated by the intensity of the line.

Recently, it has been suggested that lignin is not built up by a combinatorial polymerization of monolignols, but has a more ordered structure with a repeating unit larger than that given by the phenylpropane units [16, 17]. Thus, by a template polymerization involving cell wall proteins, oligomeric lignin fragments should be synthesized and added together in a controlled manner. Until now, however, there has been no experimental data to support this theory.

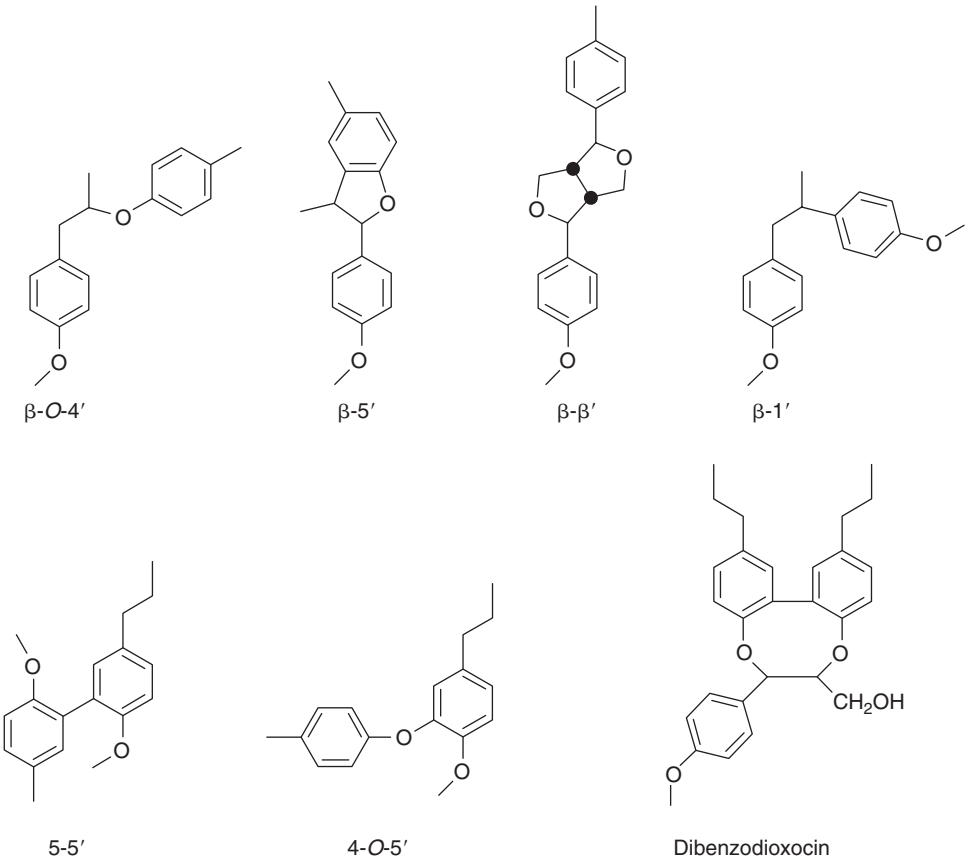

Figure 9.5 Inter-unit linkage types in lignins and their commonly used denotation.

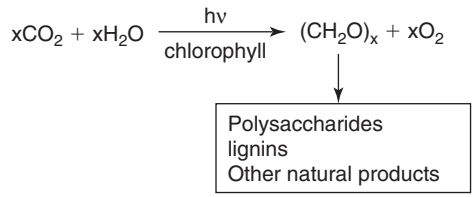

Figure 9.6 The fixation of carbon dioxide in nature through the photosynthesis reaction.

9.4 MAJOR SOURCES OF LIGNIN

Lignin is present in all vascular plants making it second to cellulose in abundance among polymers in nature. Since lignin, like many other biomass components, is formed via the photosynthesis reaction (Fig. 9.6), it is renewable and it has been estimated that the annual production of lignin on earth is in the range of 5–36 $\times$ 10^8 tons.

In woody plants from the gymnosperm and angiosperm phylum, the lignin content is in the order of 15–40 per cent [18] whereas in herbs, the lignin content is less than 15 per cent [19]. Low lignin content is usually also encountered in annual plants. In Table 9.2, some representative values for the content of lignin in various types of commercially important plants are given.

Many softwood and hardwood species, together with certain types of annual plants, have commercial interest as a source of cellulose fibres for the production of paper and board products. Thus, in technical fibre liberation processes, such as alkaline or sulphite pulping, huge quantities of lignin are dissolved as alkali lignin and lignosulphonates, respectively. With few exceptions (see Chapter 10), these lignins are, however, never isolated, but burnt

Table 9.2

Lignin content in various types of plants

Plant Scientific/Common name	Lignin content	Reference
Gymnosperms		
Picea abies, Norway spruce	28	[20]
Picea abies, Norway spruce (compression wood)	39	[21]
Pinus radiata, Monterey pine	27	[22]
Pinus sylvestris, Scots pine	28	[22]
Pseudotsuga menziesii, Douglas fir	29	[22]
Tsuga canadensis, Eastern hemlock	31	[22]
Angiosperms – Eudicotyledons		
Acasia mollissima, Black wattle	21	[22]
Betula verrucosa, Siver birch	20	[23]
Eucalyptus globulus, Blue gum eucalyptus	22	[22]
Eucalyptus grandis, Rose eucalyptus	25	[24]
Populus tremula, European aspen	19	[23]
Corchorus capsularis, Jute	13	[25]
Hibiscus cannabinus, Kenaf	12	[25]
Linun usitatissiumum, Flax	2.9	[25]
Angiosperms – Monocotyledons		
Oryza species, Rice straw	6.1	[26]
Saccharum species, Bagasse	14	[26]

together with other wood constituents liberated in the pulping liquor in order to produce the steam required for the process. In all commercial pulping processes, as well as in emerging processes, such as wood hydrolysis for the production of biofuels, the lignin is structurally altered in comparison to the native lignin. Broadly, these types of lignin can be described as being heterogeneous polyphenols with molecular masses in the range of 100–300 000, often with a high degree of polydispersity (see Section 9.6).

In growing plants, on the other hand, the lignin constitutes an integral part of the cell walls with chemical linkages to all types of polysaccharide constituents present. For spruce wood, it has been shown that the major portion of lignin is covalently linked to the hemicelluloses (*i.e.* xylan and glucomannan) with a minor amount being linked to cellulose [27]. The concentration of lignin is, however, not evenly distributed throughout the cell wall and, despite a high concentration of lignin in the middle lamella, the predominant portion is located in the S2 layer of the secondary wall due to its large relative volume [28]. A detailed analysis of the lignin distribution in two wood species, one softwood and one hardwood, has been published and is shown in Table 9.3 [29].

All attempts to isolate lignin from wood or other types of biomass must be preceded by some mechanical disintegration of the material. Usually, intensive milling of the material is employed whereby the structural integrity (*i.e.* cell types), cell layers and any inhomogeneities at the macromolecular level, is eliminated and, from such materials, only an average lignin structure can be obtained. Despite these drawbacks, almost all present knowledge about the structure of lignins is based on comprehensive milling of the plant material, followed by solvent extraction with dioxane and (sometimes) further purification to yield low to moderate yields of lignin. In most isolation procedures, the lignin contains minor impurities of carbohydrates.

9.4.1 Milled wood lignin

In 1956, the first description of the isolation of lignin from spruce wood according to these principles was published and the lignin was denoted as milled wood lignin (MWL) [30]. By employing extremely long extraction times (sequential extraction with dioxane–water, 96:4, for ~6 weeks), around 50 per cent of the lignin could be isolated. The author concluded that 'MWL is a very useful material for lignin chemists'. In a subsequent paper, several other softwood and hardwood species were used to produce the corresponding MWLs and the similarities

Table 9.3

Distribution of lignin in various cell wall layers of softwood tracheids and hardwood fibres

Wood cell	Cell wall layer	Tissue volume (%)	Lignin (% of total)	Lignin concentration (%)
Loblolly pine tracheids (softwood)				
Early wood	S1	13	12	25
	S2	60	44	20
	S3	9	9	28
	ML + P	12	21	49
	CC	6	14	64
Late wood	S1	6	6	23
	S2	80	63	18
	S3	5	6	25
	ML + P	6	14	51
	CC	3	11	78
White birch fibres (hardwood)				
	S	73	60	19
	ML + P	5	9	40
	CC	2	9	85

between various softwood lignins and the differences between hardwood lignins could be clearly demonstrated [31]. Furthermore, it was shown that a second solvent extraction could be used to provide a lignin material still containing substantial amounts of carbohydrates and this was denoted lignin carbohydrate complex (LCC) [32].

As an alternative to the isolation of lignin by solvent extraction, liquid–liquid partition of dissolved wood meal between aqueous sodium thiocyanate and benzyl alcohol has been suggested [33]. In this rather complicated procedure, it was possible to isolate about 40 per cent of the lignin in spruce wood. The lignin still contained some 10 per cent of carbohydrates. In a further alternative to the original method, the milled wood was treated with an enzyme cocktail containing cellulolytic and hemicellulolytic activities, thereby facilitating the subsequent solvent extraction. Fractions of lignin, having different amounts of remaining carbohydrates, could be isolated with a total yield of lignin of 57 per cent from spruce and 68 per cent from sweetgum respectively [34, 35]. An even higher total yield of lignin was obtained by first extracting milled spruce wood with water, followed by extraction with dioxane. After enzymatic hydrolysis of the polysaccharides present in the residue, a second extraction with dioxane was carried out affording a total yield of 84 per cent of the lignin [36]. Enzymatic hydrolysis of extensively milled wood has also been combined with acid hydrolysis employing 0.01 M hydrochloric acid. From both spruce and poplar, around 60–70 per cent of the lignin could be isolated with this method [37]. Table 9.4 gives some data on MWLs from various sources.

Almost all isolation procedures for lignin published till date have been preceded by an intensive milling of pre-extracted wood meal in a vibratory or rotatory mill. In the former type of mill, a milling time of 48 h has frequently been used [30, 34] while in the latter, about 1–2 weeks is required [36, 37]. During the milling, certain types of chemical changes take place in the lignin such as cleavage of β-*O*-4 linkages and introduction of carbonyl groups [34]. This reaction will result in the formation of new phenolic lignin end-groups with a simultaneous fragmentation of the lignin macromolecule as depicted in Fig. 9.7.

For extractives-free spruce wood, the concentration of free phenolic hydroxyl groups has been determined and values of 10–13 per 100 phenylpropane units have been obtained [38–40]. The corresponding value for spruce MWL is in the order of 20–30 (Table 9.4) depending on the intensity of milling and way of extracting the lignin. Consequently, all published analytical data on the structural composition of native lignin fail to give accurate values for the concentration of β-*O*-4 structures, since various amounts of this linkage have been mechano-chemically cleaved during the isolation procedure.

In an alternative way of isolating lignin from wood, pre-extracted spruce wood meal has been treated with a commercial mono-component endoglucanase [27, 41]. Thereby, the crystalline structure of cellulose is destroyed and the now amorphous wood can be homogeneously swollen in aqueous urea. By subsequent fractionation of the material in aqueous alkali, all lignin can be recovered as various LCCs (Table 9.5). Also in this case, however, the wood

Table 9.4

Representative data on MWL isolated from different wood species

Sample origin	OCH_3 (%)	Phenolic OH (%)	Carbohydrate (%)	Lignin yield (%)[a]	Reference
Picea abies, Norway spruce	15.45	30	1.9	19	[30]
Picea abies, Norway spruce	15.2	20	4.1	17	[34]
Picea abies, Norway spruce, CEL-96[b]	15.2	20	4.3	28	[34]
Picea mariana, Black spruce	15.41	28	n.a.[c]	n.a.	[31]
Picea mariana, Black spruce	15.3	23	<9.6	25	[37]
Picea mariana, Black spruce, EAL[d]	13.7	21	<7.7	69	[37]
Pinus sylvestris, Scots pine	15.74	27	n.a.	n.a.	[31]
Betula verrucosa, Silver birch	21.51	n.d.	7.5	n.a.	[31]
Liquidambar styraciflua, Sweetgum	21.4	14	3.6	17	[34]
Liquidambar styraciflua, Sweetgum, CEL-96[b]	21.5	13	3.8	43	[34]
Populus tremuloides, Trembling aspen	19.6	21	<8.0	24	[37]
Populus tremuloides, Trembling aspen, EAL[d]	20.9	21	<4.3	61	[37]

[a] Based on the lignin content in wood.
[b] Ball milling of wood followed by enzymatic hydrolysis of polysaccharides prior to extraction of the residue with dioxane–water (96:4).
[c] n.a. = not analysed.
[d] Ball milling of wood followed by enzymatic hydrolysis of polysaccharides and subsequent acid hydrolysis with 0.01 M HCl of the residue in dioxane–water (85:15).

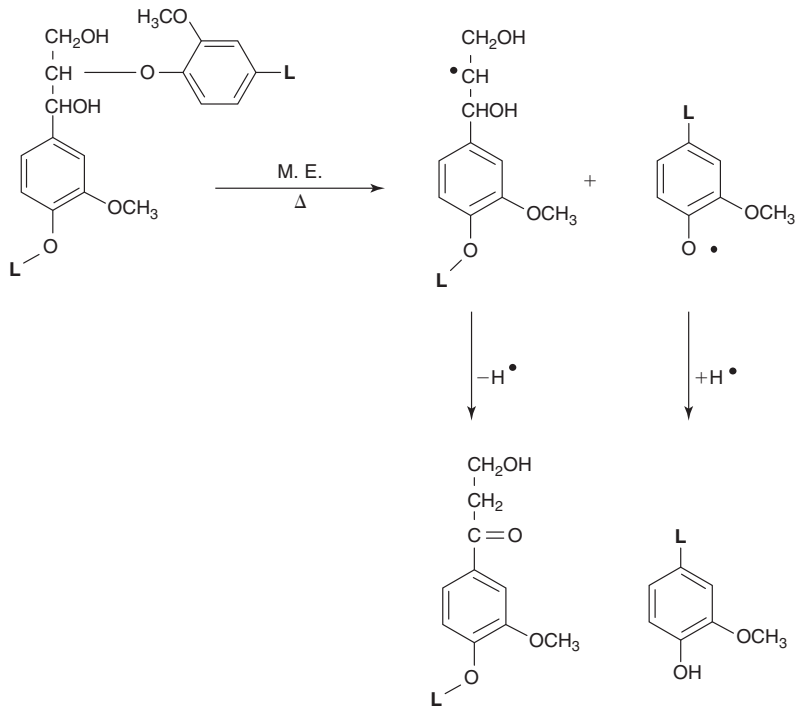

Figure 9.7 Lignin fragmentation reaction suggested to take place during the milling of wood. M.E. = mechanical energy.

Table 9.5

Types of LCC isolated from spruce wood meal after treatment with endoglucanase followed by swelling in urea and fractionation in aqueous alkali [27, 41]

Type of lignin–carbohydrate complex (LCC)	Lignin yield (%)
GalactoGlucoMannan – Lignin	8
Glucan – Lignin	4
GlucoMannan – Lignin[a]	48
Xylan – Lignin[a]	40

[a] Sum of two different fractions.

structure must be degraded to some extent prior to enzyme treatment by vibratory ball milling for 3 h and, thus, neither this lignin can be regarded as completely native although the milling intensity has been greatly reduced.

9.4.2 Dehydropolymerizate

In the early work on lignin structure, enzymatic polymerization of coniferyl alcohol, employing either laccase or peroxidase together with oxygen or hydrogen peroxide respectively, has frequently been used to produce 'lignin-like' substances denoted dehydrogenation polymers (DHPs) (see Fig. 9.4) [10]. The results from this work were used to construct the first generation of statistical lignin structures in which the then identified relative amounts of different inter-unit linkages were included (Fig. 9.8).

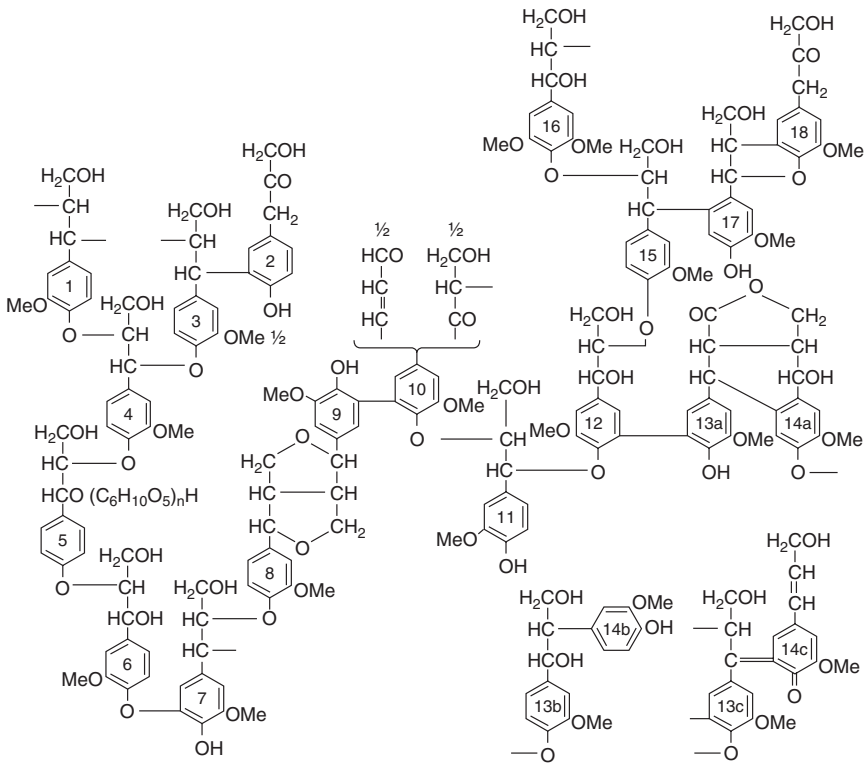

Figure 9.8 A first generation schematic formula of spruce lignin showing the most important inter-unit linkages between the phenylpropane units [42]. Reproduced by permission of Springer Science and Business Media.

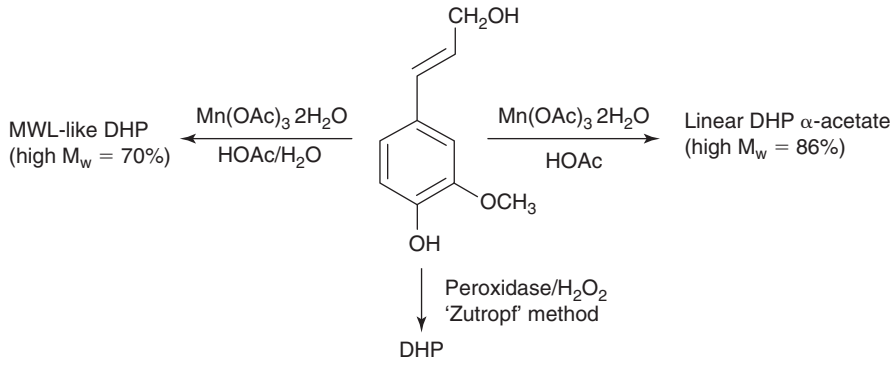

Figure 9.9 Synthesis of DHP using the 'Zutropf method' with peroxidase and hydrogen peroxide or manganese(III)-acetate in acetic acid with or without the presence of water. The biomimetic DHPs were fully acetylated and the high molecular weight fraction obtained after chromatography on polystyrene gel [46].

In the synthesis of DHP, two different modes have been employed, the 'Zulauf' and the 'Zutropf' methods. The former involves a stepwise addition of enzyme to a solution of coniferyl alcohol whereas in the latter, the reverse is used. From a structural point of view, the two methods give slightly different DHPs but the Zutropf–DHP is considered as being somewhat more 'lignin-like' [43]. For both types of DHPs, however, considerable structural differences are encountered in comparison with MWL.

Attempts to improve the synthesis of DHP in order to obtain a more lignin-like polymer have been made. In one such system, coniferin, together with β-glucosidase, peroxidase and hydrogen peroxide, prepared *in situ* from oxygen, glucose oxidase and liberated glucose, were used. Thereby, a very low concentration of hydrogen peroxide could be maintained throughout the oxidation. In different variants of the procedure, the reaction was performed with or without the presence of a polysaccharide and at different pH-values [44]. Based on this model system, it was found that pH, the concentration of monomer radicals and the carbohydrate matrix all affected the structure of the resulting DHP to some extent [45]. Despite these modifications, however, the DHPs were still found to be quite different from MWL.

In alternative ways of preparing DHP by employing biomimetic approaches, a much closer structural resemblance to MWL could be obtained. In one method, manganese(III) acetate was used to polymerize coniferyl and/or sinapyl alcohol [46]. The reaction conditions were found to exert a profound influence on the structure of the resulting polymer and lignin-like, as well as linear polymers, were produced as schematically illustrated in Fig. 9.9. In a similar approach, manganese(III) was produced *in situ* by the oxidation of manganese(II) with manganese peroxidase. Oxidation of coniferyl alcohol was carried out in a two-vessel reaction flask separated by a semi-permeable membrane allowing the manganese(III) to freely move between the vessels, while keeping the enzyme and coniferyl alcohol separated. Again, a lignin-like DHP could be formed as revealed by thioacidolysis in combination with size exclusion chromatography and ^{13}C NMR spectroscopy [47].

9.5 THE STRUCTURE OF LIGNIN

The quantification of lignin present in wood and other plants is usually done indirectly by weighing the solid residue that remains after the complete hydrolysis of all polysaccharides present in the material [48]. The insoluble material, denoted Klason lignin, is thought to be formed by a comprehensive condensation of the original lignin structure by the strongly acidic conditions used in the hydrolysis. In addition, a minor amount of acid-soluble lignin can be determined by measuring the UV absorbance of the hydrolysis liquor, usually at 205 nm [48]. Although the latter value is often added together with the value for Klason lignin to provide the total amount of lignin in a sample, its origin is not known with certainty and, for example, carbohydrates may well contribute degradation products with absorbance in the same region (Gellerstedt, unpublished).

For the quantification of lignin in a large number of similar samples, near-infrared spectroscopy (NIR) is well suited. Once a suitable set of reference samples has been determined using the Klason lignin method, the data can be used to calibrate the NIR signal. Subsequently, the lignin content in unknown samples can be registered in a convenient and rapid way by collecting the respective NIR spectra [49].

Among the early methods for the identification of lignin in a sample, colour reactions have been frequently used [50]. Thus, the treatment of wood or mechanical pulp with a mixture of phloroglucinol and hydrochloric acid results in the development of a reddish colour attributable to a condensation product between coniferaldehyde end-groups in lignin and phloroglucinol.

9.5.1 Wet chemistry methods

Today, a large number of wet chemistry methods exist by which lignin can be identified on the basis of its pattern of degradation products. Such methods have also been extensively used to get information about the structural details of different types of lignins. In Table 9.6, some commonly used methods have been collected. Each one of these methods will provide a piece of structural information but in most cases, the information is at best semi-quantitative.

Based on comprehensive work on spruce and birch MWL employing predominantly oxidation with permanganate–hydrogen peroxide and acidolysis respectively as analytical tools, a large number of degradation products has been identified and quantified [51, 56]. Based on these data, the concentration of individual inter-unit linkages present in the two lignins has been calculated and a structural scheme of spruce lignin, similar to that depicted in Fig. 9.8, was constructed [61]. An approach employing catalytic hydrogenation of spruce wood and subsequent identification of the degradation products was found to yield similar results in terms of functional groups and relative frequency of inter-unit linkages [54]. In a similar way, thioacetolysis (*i.e.* acid catalysed condensation of lignin with thioacetic acid), followed by alkaline hydrolysis and reduction with Raney nickel, has been applied on beech wood meal to degrade the lignin into a mixture of monomeric and oligomeric products. Several of these were identified and quantified providing data which formed the basis for a constitutional scheme of beech lignin [62, 63].

9.5.2 Nuclear magnetic resonance

Contrary to the wet chemical methods, analysis by nuclear magnetic resonance (NMR) does not degrade the sample and, consequently, all structural features in a lignin can be visualized. Despite the fact that analysis by either proton or carbon NMR results in crowded spectra with a severe overlap of signals, numerous spectra of lignins have been published and a large number of individual signals assigned [64–69]. By the use of spectral editing with, for example, the DEPT-sequence, permitting the separate analysis of CH-, CH_2- and CH_3-groups respectively, further details of the assignment of individual signals have been achieved [70]. Furthermore, the use of inverse gated decoupling combined with long delay times between pulses has permitted the quantitative analysis of ^{13}C NMR spectra thus providing reliable data on the amount of different types of carbon atoms in spruce MWL [71, 72].

Two-dimensional correlation spectra such as the HSQC-sequence can be used to separate the carbon and proton signals, thus permitting a much higher degree of accuracy in signal assignment. Furthermore, such spectra can be integrated in the *z*-direction to give quantitative information provided that a suitable reference signal has been chosen [73]. In Table 9.7, the result of such an integration is shown together with similar data taken from the literature and based on NMR, as well as on wet chemical methods.

Table 9.6

Wet chemistry methods for the analysis of lignin and lignin structures

Method	Reaction principle	Reference
Oxidation with $KMnO_4 - H_2O_2$	Oxidative elimination of side chains	[51, 52]
Thioacetolysis	Cleavage of alkyl-aryl ethers	[53]
Hydrogenolysis	Reductive cleavage of ethers	[54, 55]
Acidolysis	Hydrolytic cleavage of ethers	[56, 57]
Thioacidolysis	Hydrolytic cleavage of ethers	[4, 58]
Acetyl bromide – Zinc/Acetic anhydride	Reductive cleavage of ethers	[59, 60]

Table 9.7

Frequency of inter-unit linkages in MWL from spruce (*Picea abies*) based on integration of a 2D HSQC spectrum using the aromatic C2 as internal standard

Lignin structure	Number per C9-unit	Reference		
		[74][a]	[54][b]	[61][c]
β-*O*-4′	0.43	0.45	0.45	0.50
β-5′	0.12	0.09	0.14	0.12
Dibenzodioxocin	0.05	0.07		
β−β′	0.035	0.06	0.07	0.02
β-1′	0.02	0.01	0.09	0.07
Spirodienone	0.02	0.02		
Coniferyl alcohol	0.02	0.02	0.04	
Coniferaldehyde	0.03	0.04	0.04	
Vanillin	0.02	0.05	0.06	
Dihydroconiferyl alcohol	0.02	0.02	0.04	
5–5′		0.13	0.11	0.11
4-*O*-5′			0.035	0.04

[a] Based on quantitative ^{13}C NMR.
[b] Based on hydrogenolysis of ezomatsu (*Picea jezoensis*) wood.
[c] Based predominantly on permanganate–hydrogen peroxide oxidation.

9.5.3 Analysis of β-*O*-4 structures

The quantitatively most important inter-unit linkage in lignin, the β-*O*-4 linkage, can be cleaved in alkaline as well as in acidic conditions. For analytical purposes, alkaline cleavage is used as part of the thioacetolysis reaction sequence [53], whereas acidic conditions can be applied directly to the wood material [75]. In the latter method, hydrochloric acid has been used to afford monomeric and dimeric lignin degradation products which can be identified but, in addition, the reaction also gives rise to products of higher molecular mass, at least in part formed by condensation reactions [56]. Although the method can be used as an analytical tool to give a relative quantification of the number of β-*O*-4 structures, the presence of condensation reactions is a serious drawback [57]. The chemistry of lignin encountered in acid media, *viz*. the intermediate formation of a benzylic cation, followed by either a proton elimination reaction or an addition of a nucleophilic carbon atom, readily explains the observed behaviour. The presence of an external nucleophile may, however, in analogy to sulphite pulping or analysis by thioacetolysis, prevent any condensation reaction.

In thioacidolysis, a Lewis acid, boron trifluoride etherate, is used together with a strong nucleophile, ethanethiol, to hydrolyse all β-*O*-4 linkages present in a lignin, wood or pulp sample [20, 58]. The predominant product from the cleavage reaction (Fig. 9.10), a phenylpropane structure (two diastereoisomeric forms) having all side-chain carbons substituted with ethylthio groups, can be readily quantified. Furthermore, several other monomeric degradation products can also be identified, thus providing information about the variability of lignin end-groups [4].

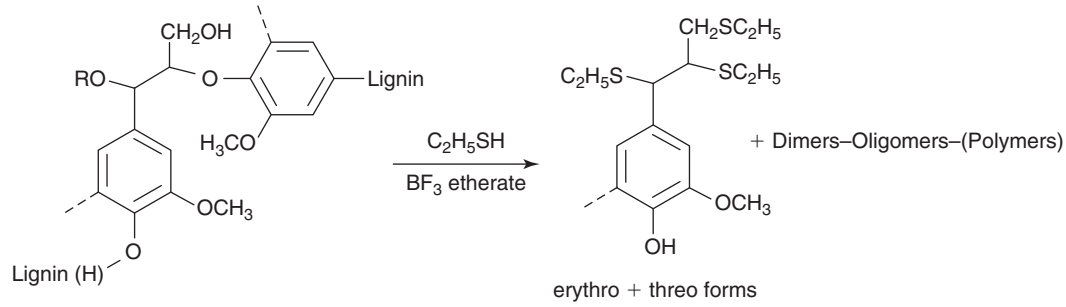

Figure 9.10 Thioacidolysis of lignin and formation of ethylthio-substituted degradation products.

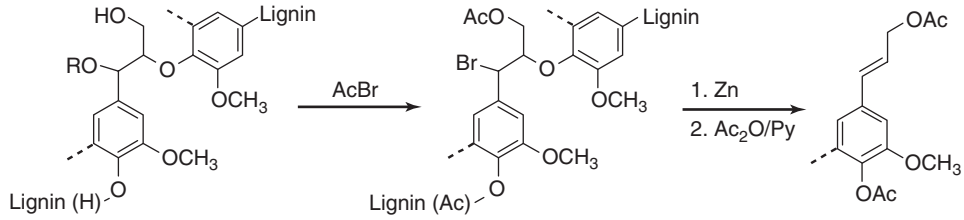

Figure 9.11 Analysis of β-*O*-4 structures in lignin according to the DFRC method (derivatization followed by reductive cleavage) resulting in the formation of cinnamyl alcohol derivatives suitable for quantification. AcBr = acetyl bromide, Ac_2O/Py = acetic anhydride/pyridine [59].

From wood, the reaction gives a high yield of low molecular mass degradation products (monomers to trimers, with traces of higher M_w products) and, after treatment, no lignin can be detected in the remaining wood material. Thioacidolysis, when applied to kraft pulps, on the other hand, in addition to monomers–trimers also results in the presence of polymeric material formed as a consequence of pulping reactions [20].

In a later extension of the thioacidolysis reaction, a second step, a Raney nickel reduction, was introduced, thereby also permitting the analysis of dimeric and trimeric degradation products by gas chromatography [20, 76]. By this method, more than 20 different dimeric and 16 trimeric structures, representing all the various inter-unit linkages depicted in Fig. 9.5, except the β-*O*-4 and dibenzodioxocin structures, have been qualitatively identified from spruce wood. Similar data from birch and aspen wood are also available [23].

A different approach to the analysis of β-*O*-4 structures involves a two-step procedure with acetyl bromide treatment followed by a reductive cleavage of the β-ether substituent as depicted in Fig. 9.11 [59, 60]. The reaction can be applied to isolated lignins, as well as on cell wall material, and results in the formation of acetylated cinnamyl alcohols which can be readily quantified by gas chromatography. The yield of the major compounds originating from G-, H- and S-units seems, however, to be inferior to the corresponding values obtained from thioacidolysis [20, 60, 76].

Thioacidolysis results in a high yield of the major monomeric degradation product (in two diastereoisomeric forms) from softwoods, as well as from hardwoods. In addition, it is possible to identify several other monomers in minor amounts (Fig. 9.2) [4]. The latter are assumed to constitute lignin end-groups attached to the rest of the polymer through β-*O*-4′ linkages. By pre-swelling of the wood in water prior to the thioacidolysis reaction, an apparent quantitative delignification can be achieved and the yield of the major monomer(s) substantially increased, as shown in Table 9.8. From this table, it is also obvious that the milling used to extract MWL from the wood samples

Table 9.8

Yield of the main monomer(s) on thioacidolysis of spruce, birch and aspen wood, spruce TMP and the corresponding MWLs together with the number of phenolic hydroxyl groups per 100 phenylpropane (C9) units [20, 23]

Sample	Yield of the main monomer(s) (μmol/g Klason lignin)	Content of phenolic OH[a], Number per 100 C9-units[b]
Spruce wood	1 332	n.a.[c]
Spruce wood (pre-swollen)[d]	1 682 (31%)[e]	13
Spruce MWL[f]	986	20
Spruce TMP[g] (pre-swollen)[d]	1 498	14
Birch wood (pre-swollen)[d]	672 (G) + 2 318 (S) = 2 990 (63%)[e]	7.6
Birch MWL[f]	403 (G) + 809 (S) = 1212	n.a.[c]
Aspen wood (pre-swollen)[d]	866 (G) + 1 942 (S) = 2 808 (58%)[e]	10
Aspen MWL[f]	609 (G) + 863 (S) = 1 472	n.a.[c]

[a] Determination by periodate oxidation according to [40].

[b] Assumed molecular mass ($g\,mol^{-1}$) for one C9-unit: spruce = 183; birch = 210; aspen = 206.

[c] n.a. = not analysed.

[d] Sample pre-swollen in water over-night.

[e] Percentage of the theoretical yield of C9-units per gram Klason lignin.

[f] Yield given as $\mu molg^{-1}$ MWL.

[g] Thermomechanical pulp.

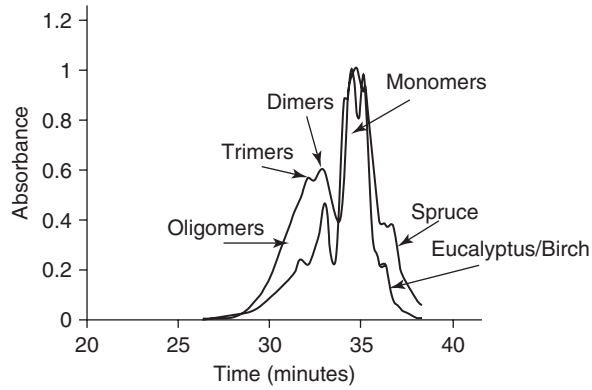

Figure 9.12 HPSEC on styragel of the acetylated lignin degradation products obtained after thioacidolysis of spruce, birch and eucalyptus wood respectively.

results in substantially reduced amounts of monomeric thioacidolysis products, as discussed above (see Figure 9.7). Simultaneously, the number of phenolic hydroxyl groups is strongly increased. Even the comparatively mild milling procedure used in the production of thermomechanical pulp (TMP), results in an increased number of phenolic hydroxyl groups and a reduction in monomer yield. In the two hardwood samples shown in Table 9.8, the yield of S-units is high and reflects the fact that β-*O*-4′ structures are completely dominant in syringyl type lignins, since the β-5′ and 5-5′ coupling modes cannot take place. Generally, the content of phenolic hydroxyl groups in hardwoods is lower as compared to softwoods; a result in line with a high abundance of linear β-*O*-4′-linked syringyl lignin. In guaiacyl lignins, on the other hand, β-*O*-4′ moieties may be present in both linear and branched/crosslinked structures, thus resulting in a considerably lower yield of the monomeric thioacidolysis product.

When applied to wood, the thioacidolysis reaction seems to result in a complete dissolution of lignin, since no Klason lignin can be detected in the residue. After acetylation, the degraded lignin fragments can be conveniently analysed by high performance size exclusion chromatography (HPSEC) in tetrahydrofuran [20]. From spruce, as well as from birch and eucalyptus wood, similar chromatograms can be obtained, although the relative intensity of the various peaks is different (Fig. 9.12) (Gellerstedt, unpublished). By the use of calibration substances, it can be shown that no polymeric material is present in the degradation mixture with monomers, dimers and trimers constituting the predominant types of products.

This result is completely in line with the view that the lignin polymer in both softwoods and hardwoods is built up with β-*O*-4′ linkages as the completely dominating type of inter-unit linkage. The number of such linkages has been estimated based on analytical data from MWL, and values of about 50 and 60 per 100 phenylpropane units for spruce and birch, respectively, have been obtained [77]. Obviously, these values must be much higher for the native lignin in wood since, as discussed above, the milling procedure used to produce MWL involves a comprehensive cleavage of β-*O*-4′ linkages (see Fig. 9.7 and Table 9.8).

9.5.4 Lignin heterogeneity

By a selective modification of the cellulose structure in spruce wood, by treatment with an endoglucanase, followed by swelling of the wood in aqueous urea and dissolution in alkali, a subsequent stepwise acidification will result in a complete recovery of all the lignin as different LCCs (see Table 9.5) [27,41]. On thioacidolysis followed by HPSEC of the various LCCs, large differences in the resulting lignin chromatograms were encountered, as demonstrated in Fig. 9.13.

Thus, the thioacidolysis mixture obtained from xylan-linked lignin showed a complete dominance of monomers with only a small amount of dimers present. The glucomannan lignin complex, on the other hand, gave a chromatogram similar to that obtained from wood (Fig. 9.12). A reasonable explanation for these results is the presence of two distinctly different types of lignin in spruce wood; one having a very strong dominance of β-*O*-4′ linkages, being linked to xylan and with a rather linear structure, the other being linked to glucomannan and having a more complex structure comprising the various linkages depicted in Fig. 9.5. The former type of lignin

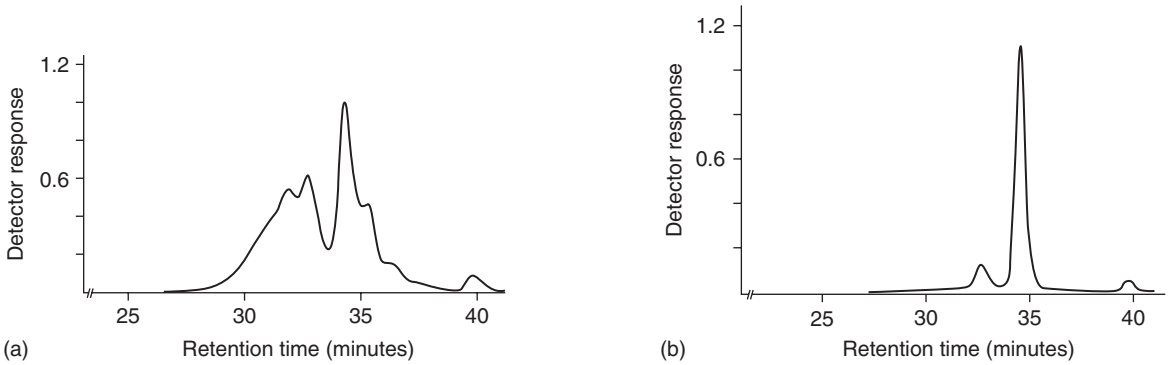

Figure 9.13 HPSEC of thioacidolysis products obtained from (a) glucomannan-linked lignin, and (b) xylan-linked lignin [41].

Table 9.9

Distribution of guaiacyl and syringyl lignin in various morphological regions of white birch (*Betula papyrifera*) [78]

Morphological differentiation	Guaiacyl/Syringyl
Fibre, S2-layer	12:88
Vessel, S2-layer	88:12
Ray parenchyma, S-layer	49:51
Middle lamella (fibre–fibre)	91:9
Middle lamella (fibre–vessel)	80:20
Middle lamella (fibre–ray)	100:0
Middle lamella (ray–ray)	88:12

should be expected to be particularly sensitive to milling, resulting in a low yield of thioacidolysis products with a corresponding high amount of free phenolic hydroxyl groups. This has been found to be the case, thus lending further support to the view that a linear β-*O*-4′-linked lignin is present in spruce wood [41].

For birch wood, it was shown a long time ago, by the use of UV microscopy, that the structure of lignin is not uniform throughout the fibre and vessel elements. Whereas the secondary wall in the fibre fraction was found to contain an almost pure syringyl lignin (*i.e.* a linear lignin with a predominance of β-*O*-4′ linkages) the lignin present in vessels was of the guaiacyl type. Guaiacyl lignin was also found in the middle lamella region [29]. In a further development of the analytical method, more precise data for the presence of G- and S-units throughout the cell walls were obtained (Table 9.9) [78].

By the use of an immunogold labelling technique with specific response for condensed guaiacyl and guaiacyl–syringyl lignin and for non-condensed guaiacyl–syringyl lignin respectively, the data in Table 9.9 have recently been qualitatively confirmed in transmission electron microscopy studies of aspen wood [79]. Further support for the presence of linear lignin structures of the β-*O*-4′ type present in hardwood has also been found by the mass spectrometric analysis of a low molecular mass fraction of eucalyptus lignin, showing the arrangement of S (and G)-units in pentamer and hexamer fragments (Fig. 9.14) [80].

9.5.5 The structure of native lignin

The growing awareness that the structure of lignin inside the fibre wall is not uniform, but probably adapted to its site of formation, chemical surrounding and other as yet unknown factors makes the presentation of statistical formulas based on MWL-data alone highly uncertain. The presence of a substantial amount of lignin in spruce, as well as in various hardwood species strongly enriched in β-*O*-4′ structures suggests, however, that linear lignin macromolecules are abundant in both softwood and hardwood lignin. These, as well as other types of lignin macromolecules,

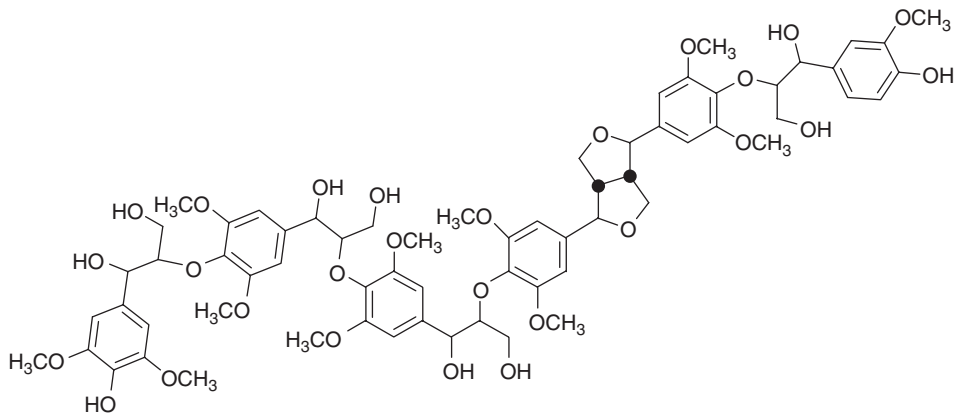

Figure 9.14 A hexameric lignin fragment from eucalyptus (*E. grandis*) identified by MS-analysis. Further pentamer and hexamer fragments were also found [80].

seem to be chemically linked to the polysaccharides thus forming network structures in the wood tissue. In technical processes, the different types of lignin macromolecules will show different reactivity, thus resulting in inhomogeneous dissolution at the nano-scale [81–83].

The knowledge collected till date on the structure of lignin distinguishes two different types of macromolecules; *viz*. a linear structure predominantly built up by β-*O*-4′ linkages in G- as well as in S-units, with minor amounts of β−β′, β-5′ and β-1′ linkages present. The number of 5-5′ and 4-*O*-5′ linkages in such lignin is assumed to be very low or zero. As a consequence, the number of free phenolic hydroxyl groups is also very low. In the second type of lignin, a much more branched structure should prevail, 5-5′ and 4-*O*-5′ linkages being present and the number of phenolic hydroxyl groups quite high. In spruce, this type of lignin should be linked predominantly to glucomannan. The presence of such a lignin requires the formation of chain-like fragments which subsequently are linked together by a secondary formation of 5-5′ and 4-*O*-5′ linkages (see Section 9.3). The variability of the chain-like fragments can be substantial, thus resulting in lignin macromolecules of considerable difference from each other. Still, the number of β-*O*-4′ linkages must be high, however, in order to fulfil all structural requirements concluded from present knowledge. A segment of such lignin is shown in Fig. 9.15.

9.6 TECHNICAL LIGNINS

For a more comprehensive discussion of technical lignins, the reader should refer to Chapter 10.

9.6.1 Kraft lignins

Delignification of wood and other biomass for the production of kraft pulp involves treatment at high temperature with an aqueous solution of sodium hydroxide and sodium sulphide. Under these conditions, most of the β-*O*-4′ structures in lignin are hydrolysed (>95 per cent) and the resulting lignin fragments dissolved in the alkaline solution [84]. Several other degradative lignin reactions also take place under the harsh conditions prevailing in the digester and most of the phenylpropane side-chains are in part eliminated, in part modified. The process results in the dissolution of around 90–95 per cent of all lignin present in the starting material. By acidification of the pulping liquor, the dissolved lignin can be recovered to a great extent as a complex mixture of phenolic structures with molecular mass in the range of ~150–200 000 [85–89].

By solvent fractionation, it has been shown that a predominant portion of softwood kraft lignin is of low molecular mass with a low degree of polydispersity as shown in Table 9.10. The remainder, on the other hand, has both high molecular mass and high polydispersity, presumably due to the presence of both polysaccharides and condensed lignin structures in these fractions [88]. A similar fractionation technique applied to a birch kraft lignin gave the results shown in Table 9.11, again with a high molecular mass tail resulting in a high degree of polydispersity for one of the fractions [89].

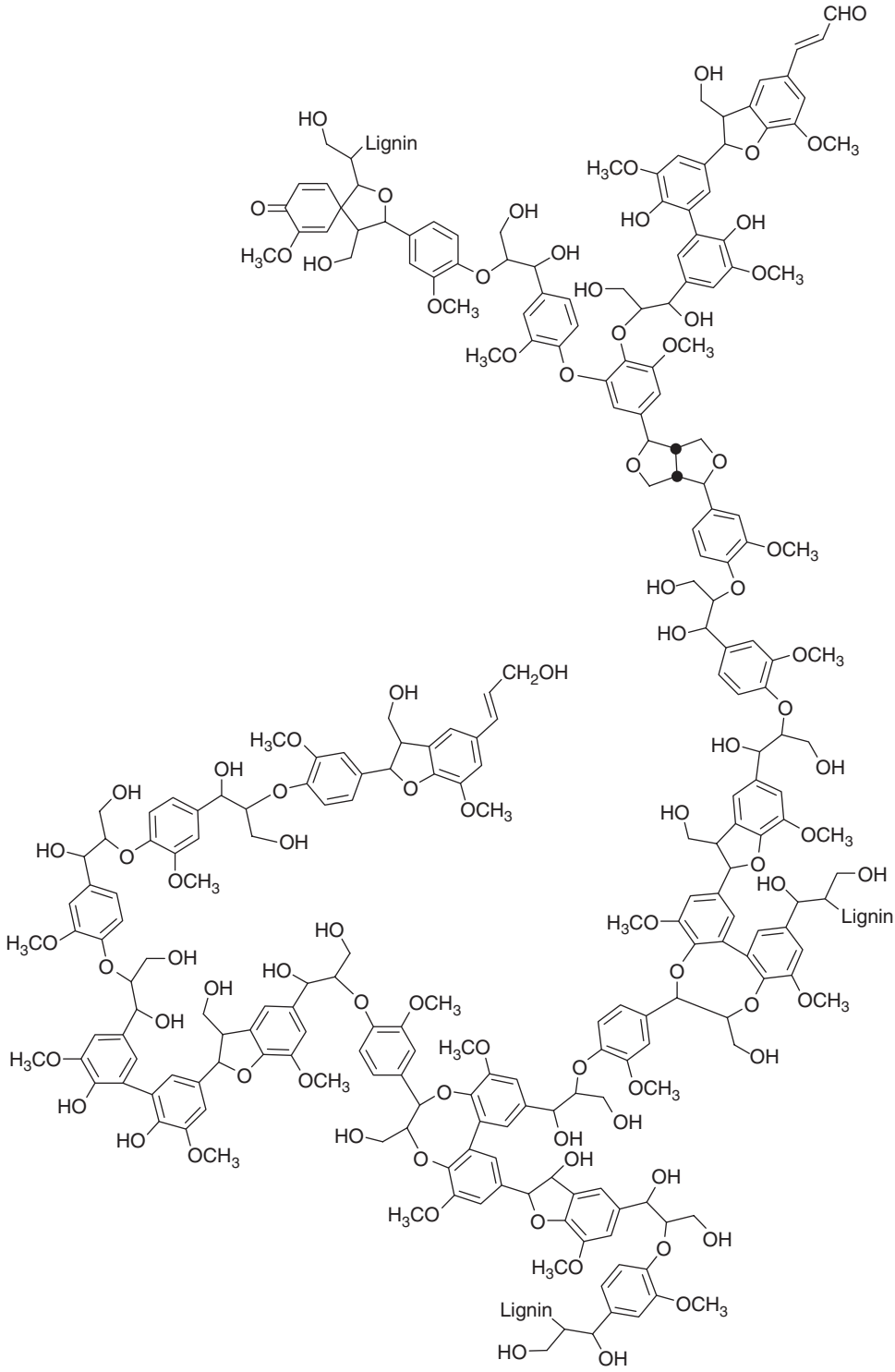

Figure 9.15 Statistical scheme of the glucomannan-linked lignin from spruce. The structure contains 25 phenylpropane units with a total of 4 free phenolic hydroxyl groups. The proportion of inter-unit lignin linkages is: 12 β-*O*-4′ (2 in dibenzodioxocin), 5 β-5′, 4 5-5′ (2 in dibenzodioxocin), 1 4-*O*-5′, 1 β−β′, 1 β-1′, 1 coniferaldehyde, 1 coniferyl alcohol.

Table 9.10

Analytical data on kraft lignins obtained after solvent fractionation of isolated industrial softwood black liquor lignin [88]

Fraction No[a]	Yield (%)	M_n[b]	M_w[b]	M_w/M_n	Phenolic OH (mmol/g)	Aliphatic OH (mmol/g)	Carboxyl (mmol/g)
1	9	450	620	1.4	5.1	1.0	2.3
2	22	900	1 290	1.4	5.0	2.3	1.1
3	26	1 710	2 890	1.7	4.3	2.1	0.8
4	28	3 800	82 000	22	3.9	2.4	0.4
5	14	5 800	180 000	31	3.0	2.4	0.3

[a] Fraction 1 = soluble in methylene chloride.
Fraction 2 = residue soluble in *n*-propanol.
Fraction 3 = residue soluble in methanol.
Fraction 4 = residue soluble in methanol/methylene chloride (7:3).
Fraction 5 = residue.
[b] Acetylated lignin fractions.

Table 9.11

Analytical data on kraft lignins obtained after solvent fractionation of isolated industrial birch black liquor lignin [89]

Fraction no[a]	Yield (%)	M_n[b]	M_w[b]	M_w/M_n	Phenolic OH (mmol/g)	Aliphatic OH (mmol/g)
1	32	650	910	1.4	5.0	1.0
2	38	1 320	2 110	1.6	4.4	2.2
3	30	3 760	87 080	23	2.9	3.7

[a] Fraction 1 = soluble in methylene chloride.
Fraction 2 = residue soluble in methanol.
Fraction 3 = residue.
[b] Acetylated lignin fractions.

Table 9.12

Elemental and methoxyl analysis data for softwood kraft lignin fractions from Table 9.10 together with data for spruce MWL and purified pine kraft lignin [88]

Fraction no	Carbon (%)	Hydrogen (%)	Oxygen (%)	Sulphur (%)	OCH_3 (%)	Sugars (%)
1	67.8	6.43	23.0	2.7	15.4	–
2	65.3	6.48	26.8	1.4	16.0	–
3	64.7	5.96	27.7	1.5	14.9	–
4	64.5	5.73	28.2	1.3	13.9	0.3
5	59.0	5.54	32.8	1.2	11.3	8.7
MWL, spruce[a]	63.8	6.0	29.7	–	15.8	
Kraft lignin[b]	64.3	6.0	27.9	1.8	13.8	0.9

[a] Reference [30].
[b] Reference [91].

NMR analysis of the various lignin fractions from pine/spruce and birch respectively [88, 89], as well as of unfractionated kraft lignin from pine [71], has shown that comprehensive changes of the side-chain structure take place during pulping with a strong reduction of oxygen-linked carbons and a concomitant increase of aliphatic methine, methylene and methyl groups. The content of aromatic rings with free phenolic hydroxyl groups is high, in particular in the low molecular mass fractions. The presence of carboxyl groups (in softwood) is substantial and can be explained by radical coupling reactions between certain fatty acids, present among the wood extractives, and phenolic end-groups in the lignin [90]. Moreover, all the lignin fractions shown in Table 9.10 also contain considerable amounts of sulphur as shown in Table 9.12. Similar data were also found for the birch lignin fractions.

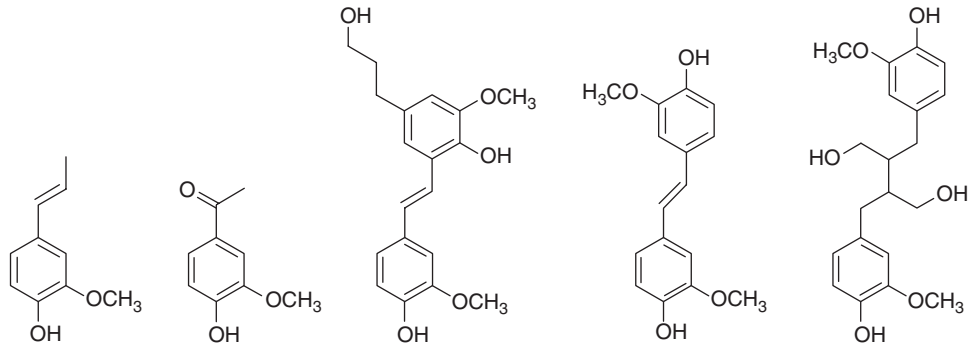

Figure 9.16 Some representative lignin fragments present in spruce kraft pulping liquor.

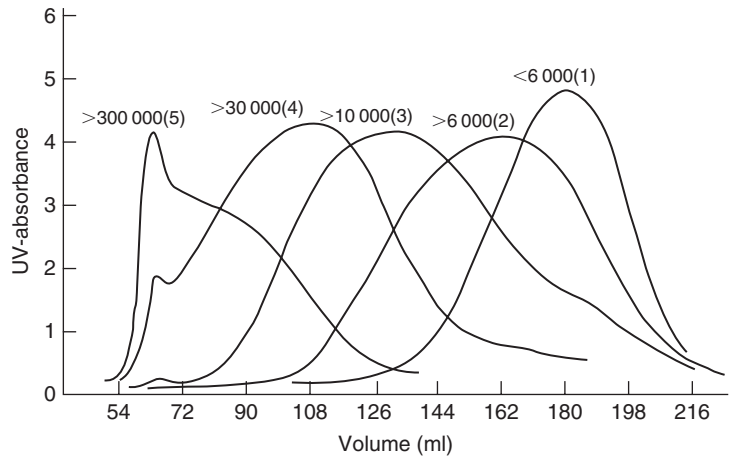

Figure 9.17 Size exclusion chromatography of lignosulphonate fractions after ultrafiltration. Fraction number and approximate molecular mass are shown in the figure [92].

Several hundred low molecular mass lignin-derived components containing one or two aromatic rings have been identified in kraft pulping liquors from spruce and birch respectively [86, 87]. Some prominent structures are shown in Fig. 9.16.

9.6.2 Sulphite lignin

The major reaction in sulphite pulping involves the introduction of sulphonate groups in the C_α-positions in lignin via the intermediate formation of carbo-cations. Thereby, any lignin–carbohydrate linkages present in these positions are cleaved and, together with some hydrolytic cleavage of β-*O*-4′ linkages, the lignin is solubilized. Condensation, predominantly between C_α and C_6 in adjacent lignin structures, is a major competing reaction, but can be suppressed by having a high concentration of bisulphite ions present in the pulping liquor. The highly water-soluble lignosulphonate can be purified from other substances present in the pulping liquor by ultrafiltration and by using membranes having different M_w cut-off values, a series of lignosulphonate fractions can be obtained as shown in Fig. 9.17 [92]. By elemental and methoxyl analysis on these fractions, a degree of sulphonation of about 0.4–0.5 per phenylpropane unit has been found. The liberation of phenolic hydroxyl groups, on the other hand, is limited but clearly visible in those lignosulphonate fractions having a low molecular mass distribution (Table 9.13). In comparison with kraft lignins, the side-chains in lignosulphonates are still intact to a high degree, as revealed by ^{13}C NMR spectroscopy, although some α-carbon atoms are linked in condensation reactions [93].

Table 9.13

Analytical data of lignosulphonate fractions after ultrafiltration of a technical softwood acid sulphite pulping liquor [92]

Fraction no	Yield (%)	M_w	Phenolic OH (mmol/g)	Sulphonate/Phenylpropane
1	20	590	2.01	0.48
2	29	1 440	0.91	0.52
3	18	3 690	0.65	0.43
4	4	6 500	0.50	0.40
5	6	10 880	0.49	0.39
6	22	21 950	0.51	0.36

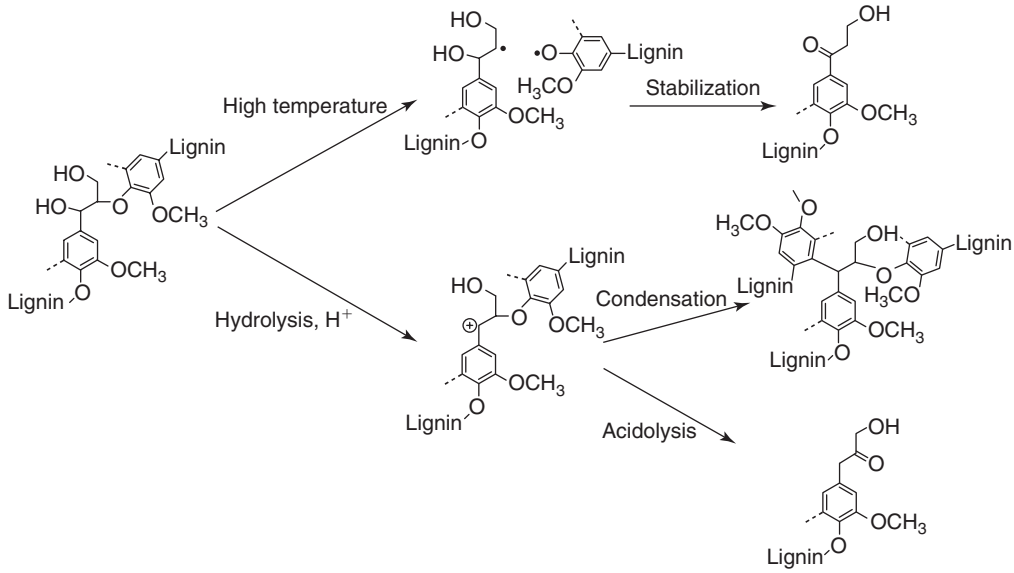

Figure 9.18 Types of lignin reactions encountered during steam explosion. The relative importance may differ depending on the steam treatment conditions.

9.6.3 Steam explosion lignin

By subjecting wood or other biomass to high temperature steam treatment, followed by a rapid pressure release, the fibrous mass is 'exploded' and liberated fibres together with fibre bundles are formed. By adjusting the time and temperature, different degrees of wood polymer modification and degradation can be achieved. Although the process is not commercial at present, it has gained much attention as a possible means for simple and cheap separation of wood polymers (*e.g.* for the production of micro-crystalline cellulose and bio-based ethanol). In particular, hardwood species such as aspen are suitable raw materials, since the lignin portion can be extracted, to a large extent, by either aqueous alkali or by organic solvents leaving a residue highly enriched in cellulose [94, 95].

In a pure steam explosion process without any added chemicals, the reaction conditions are weakly acidic, due to the release of acetic acid from the hemicellulose. Thus, the major reaction types are similar to those present in acidic sulphite pulping, *viz.* hydrolysis of polysaccharides and hydrolysis and condensation of lignin. In addition, due to the high temperature usually employed (~200°C), homolytic cleavage reactions of, for example, β-*O*-4′ linkages in lignin can be assumed to take place. The reaction types for lignin are summarized in Fig. 9.18. Altogether, these reactions result in a highly heterogeneous lignin structure containing both degraded lignin fragments and recombined fragments through condensation reactions [96]. Consequently, the number of β-*O*-4′ structures is much lower, as compared with that of the starting material and the content of phenolic end-groups higher. In addition, the number of carbonyl groups is considerably increased due to hydrolytic and/or homolytic cleavage reactions.

REFERENCES

1. Fengel D., Wegener G., *Wood. Chemistry, Ultrastructure, Reactions*, Walter de Gruyter, Berlin, 1984. pp. 132–181.
2. Sjöström E., *Wood Chemistry. Fundamentals and Applications*, 2nd Edition, Academic Press Inc., San Diego, USA, 1993. pp. 71–91.
3. Sakakibara A., Sano Y., Chemistry of lignin, in *Wood and Cellulosic Chemistry* (Eds. Hon D.N.-S. and Shiraishi N.), 2nd Edition, Marcel Dekker Inc., New York, USA, 2001, pp. 109–174.
4. Rolando C., Monties B., Lapierre C., Thioacidolysis, in *Methods in Lignin Chemistry* (Eds. Lin S.Y. and Dence C.W.), Springer-Verlag, Berlin Heidelberg, Germany, 1992, pp. 334–349.
5. Ralph J., Lu F., The DFRC method for lignin analysis. 6. A simple modification for identifying natural acetates on lignins, *J. Agr. Food Chem.*, **46**, 1998, 4616–4619.
6. Chen C.-L., Nitrobenzene and cupric oxide oxidations, in *Methods in Lignin Chemistry* (Eds. Lin S.Y. and Dence C.W.), Springer-Verlag, Berlin Heidelberg, Germany, 1992, p. 301.
7. Higuchi T., Lignin biochemistry: Biosynthesis and biodegradation, *Wood Sci. Technol.*, **24**, 1990, 23–63.
8. Boudet A.-M., Lignins and lignification: Selected issues, *Plant Physiol. Biochem.*, **38**, 2000, 81–96.
9. Donaldson L.A., Lignification and lignin topochemistry – An ultrastructural view, *Phytochemistry*, **57**, 2001, 859–873.
10. Freudenberg K., in *Constitution and Biosynthesis of Lignin* (Eds. Freudenberg K. and Neish A.C.), Springer-Verlag, Berlin-Heidelberg, 1968, pp. 47–122.
11. Katayama Y., Fukuzumi T., Enzymatic synthesis of three lignin-related dimers by an improved peroxidase–hydrogen peroxide system, *Mokuzai Gakkaishi*, **24**, 1978, 664–667.
12. Ralph J., Lundquist K., Brunow G., Lu F., Kim H., Schatz P.F., Marita J.M., Hatfield R.D., Ralph S.A., Christensen J.H., Boerjan W., Lignins: Natural polymers from oxidative coupling of 4-hydroxyphenylpropanoids, *Phytochemistry Rev.*, **3**, 2004, 29–60.
13. Zhang L., Gellerstedt G., Ralph J., Lu F., NMR studies on the occurrence of spirodienone structures in lignins, *J. Wood Chem. Technol.*, **26**, 2006, 65–79.
14. Karhunen P., Rummakko P., Sipilä J., Brunow G., Kilpeläinen I., Dibenzodioxocins; a novel type of linkage in softwood lignins, *Tetrahedron Lett.*, **36**, 1995, 169–170.
15. Argyropoulos D.S., Jurasek L., Kristofova L., Xia Z., Dun Y., Palus E., Abundance and reactivity of dibenzodioxocins in softwood lignin, *J. Agr. Food Chem.*, **50**, 2002, 658–666.
16. Lewis N.G., A 20th century roller coaster ride: A short account of lignification, *Current Opin. Plant Biol.*, **2**, 1999, 153–162.
17. Davin L.B., Lewis N.G., Lignin primary structure and dirigent sites, *Current Opin. Biotechnol.*, **16**, 2005, 407–415.
18. Fengel D., Wegener G., *Wood. Chemistry, Ultrastructure, Reactions*, Walter de Gruyter, Berlin, 1984. pp. 56–58
19. dos Santos Abreu H., Maria M.A., Reis J.L., Dual oxidation ways toward lignin evolution, *Floresta e Ambiente*, **8**, 2001, 207–210.
20. Önnerud H., Gellerstedt G., Inhomogeneities in the chemical structure of spruce lignin, *Holzforschung*, **57**, 2003, 165–170.
21. Önnerud H., Lignin structures in normal and compression wood. Evaluation by thioacidolysis using ethanethiol and methanethiol, *Holzforschung*, **57**, 2003, 377–384.
22. Sjöström E., *Wood Chemistry. Fundamentals and Applications*, 2nd Edition, Academic Press Inc., San Diego, USA, 1993, p. 249.
23. Önnerud H., Gellerstedt G., Inhomogeneities in the chemical structure of hardwood lignins, *Holzforschung*, **57**, 2003, 255–265.
24. Bassa A., Sacon V.M., da Silva Junior F.G. and Barrichelo L.E.G. Polpacao kraft convencional e modificada para madeiras de Eucalyptus grandis e hibrido (E. grandis x E. urophylla), *35° Congresso e Exposicao Anual de Celulose e Papel*, Sao Paulo, Brazil, October 14–17, 2002, Proceedings, p. 5.
25. del Rio J.C., Rodriguez I.M. and Gutierrez A. Chemical characterization of fibers from herbaceous plants commonly used for manufacturing of high quality paper pulps. *9th European Workshop on Lignocellulosics and Pulp*, Vienna, Austria, August, 27–30, 2006, Proceedings, pp. 109–112.
26. http://www.vcn.vnn.vn/sp_pape/spec_5_4_2001_13.htm.
27. Lawoko M., Henriksson G., Gellerstedt G., Characterization of lignin–carbohydrate complexes (LCCs) of spruce wood (*Picea abies* L.) isolated with two methods, *Holzforschung*, **60**, 2006, 156–161.
28. Fergus B.J., Procter A.R., Scott J.A.N., Goring D.A.I., The distribution of lignin in sprucewood as determined by ultraviolet microscopy, *Wood Sci. Technol.*, **3**, 1969, 117–138.
29. Fergus B.J., Goring D.A.I., The distribution of lignin in birch wood as determined by ultraviolet microscopy, *Holzforschung*, **24**, 1970, 118–124.
30. Björkman A., Studies on finely divided wood. Part 1. Extraction of lignin with neutral solvents, *Svensk Papperstidn.*, **59**, 1956, 477–485.
31. Björkman A., Person B., Studies on finely divided wood. Part 2. The properties of lignins extracted with neutral solvents from softwoods and hardwoods, *Svensk Papperstidn*, **60**, 1957, 158–169.

32. Björkman A., Studies on finely divided wood. Part 3. Extraction of lignin–carbohydrate complexes with neutral solvents, *Svensk Papperstidn*, **60**, 1957, 243–251.
33. Brownell H.H., Isolation of milled wood lignin and lignin–carbohydrate complex, *Tappi*, **48**(9), 1965, 513–519.
34. Chang H-m., Cowling E.B., Brown W., Adler E., Miksche G., Comparative studies on cellulolytic enzyme lignin and milled wood lignin of sweetgum and spruce, *Holzforschung*, **29**, 1975, 153–159.
35. Glasser W.G., Barnett C.A., The structure of lignins in pulps. II. A comparative evaluation of isolation methods, *Holzforschung*, **33**, 1979, 78–86.
36. Bezuch B., Polcin J., The structural study of milled wood and enzymatically isolated lignin fractions from spruce wood, *Cell. Chem. Technol.*, **12**, 1978, 473–482.
37. Wu S., Argyropoulos D.S., An improved method for isolating lignin in high yield and purity, *J. Pulp Pap. Sci.*, **29**, 2003, 235–240.
38. Yang J.-M., Goring D.A.I., The phenolic hydroxyl content of lignin in spruce wood, *Can. J. Chem.*, **58**, 1980, 2411–2414.
39. Gellerstedt G., Lindfors E.-L., Structural changes in lignin during kraft cooking. Part 4. Phenolic hydroxyl groups in wood and kraft pulps, *Svensk Papperstidn*, **87**, 1984, R115–R118.
40. Lai Y.-Z., Guo X.-P., Variation of the phenolic hydroxyl group content in wood lignins, *Wood Sci. Technol.*, **25**, 1991, 467–472.
41. Lawoko M., Henriksson G., Gellerstedt G., Structural differences between the lignin–carbohydrate complexes present in wood and in chemical pulps, *Biomacromolecules*, **6**, 2005, 3467–3473.
42. Freudenberg K., in *Constitution and Biosynthesis of Lignin* (Eds. Freudenberg K. and Neish A.C.), Springer-Verlag, Berlin-Heidelberg, 1968, p. 103.
43. Nimz H., Mogharab I., Ludemann H.-D., ^{13}C-Kernresonanzspektren von Ligninen, 3. Vergleich von Fichtenlignin mit kunstlichem Lignin nach Freudenberg, *Macromol. Chem.*, **175**, 1974, 2563–2575.
44. Terashima N., Atalla R.H., Ralph S.A., Landucci L.L., Lapierre C., Monties B., New preparations of lignin polymer models under conditions that approximate cell wall lignification. I. Synthesis of novel lignin polymer models and their structural characterization by ^{13}C NMR, *Holzforschung*, **49**, 1995, 521–527.
45. Terashima N., Atalla R.H., Ralph S.A., Landucci L.L., Lapierre C., Monties B., New preparations of lignin polymer models under conditions that approximate cell wall lignification. II. Structural characterization of the models by thioacidolysis, *Holzforschung*, **50**, 1996, 9–14.
46. Landucci L.L., Reaction of *p*-hydroxycinnamyl alcohols with transition metal salts. 3. Preparation and NMR characterization of improved DHPs, *J. Wood Chem. Technol.*, **20**, 2000, 243–264.
47. Önnerud H., Zhang L., Gellerstedt G., Henriksson G., Polymerization of monolignols by redox shuttle-mediated enzymatic oxidation: A new model in lignin biosynthesis, *Plant Cell*, **14**, 2002, 1953–1962.
48. Dence C.W., The determination of lignin, in *Methods in Lignin Chemistry* (Eds. Lin S.Y. and Dence C.W.), Springer-Verlag, Berlin Heidelberg, Germany, 1992, pp. 33–61.
49. Wallbäcks L., Edlund U., Norden B., Berglund I., Multivariate characterization of pulp using solid-state carbon-13 NMR, FTIR and NIR, *Tappi J.*, **74**(10), 1991, 201–206.
50. Nakano J., Meshitsuka G., The detection of lignin, in *Methods in Lignin Chemistry* (Eds. Lin S.Y. and Dence C.W.), Springer-Verlag, Berlin Heidelberg, Germany, 1992, pp. 23–32.
51. Erickson M., Larsson S., Miksche G.E., Gaschromatografische Analyse von Ligninoxydationsprodukten. VII. Ein verbessertes Verfahren zur Charakterisierung von Ligninen durch Methylierung und oxydativen Abbau, *Acta Chem. Scand.*, **27**, 1973, 127–140.
52. Gellerstedt G., Permanganate oxidation, in *Methods in Lignin Chemistry* (Eds. Lin S.Y. and Dence C.W.), Springer-Verlag, Berlin Heidelberg, Germany, 1992, pp. 322–333.
53. Nimz H., Uber ein neues Abbauverfahren des Lignins, *Chem. Ber.*, **102**, 1969, 799–810.
54. Sakakibara A., A structural model of softwood lignin, *Wood Sci. Technol.*, **14**, 1980, 89–100.
55. Sakakibara A., Hydrogenolysis, in *Methods in Lignin Chemistry* (Eds. Lin S.Y. and Dence C.W.), Springer-Verlag, Berlin Heidelberg, 1992, pp. 350–368.
56. Lundquist K., Low-molecular weight lignin hydrolysis products, *Appl. Polym. Symp.*, **28**, 1976, 1393–1407.
57. Lapierre C., Rolando C., Monties B., Characterization of poplar lignins acidolysis products: Capillary gas–liquid and liquid–liquid chromatography of monomeric compounds, *Holzforschung*, **37**, 1983, 189–198.
58. Lapierre C., Monties B., Rolando C., Thioacidolysis of lignin: Comparison with acidolysis, *J. Wood Chem. Technol.*, **5**, 1985, 277–292.
59. Lu F., Ralph J., Derivatization followed by reductive cleavage (DFRC method), a new method for lignin analysis: Protocol for analysis of DFRC monomers, *J. Agr. Food Chem.*, **45**, 1997, 2590–2592.
60. Lu F., Ralph J., The DFRC method for lignin analysis. 2. Monomers from isolated lignins, *J. Agr. Food Chem.*, **46**, 1998, 547–552.

61. Adler E., Lignin chemistry – Past, present and future, *Wood Sci. Technol.*, **11**, 1977, 169–218.
62. Nimz H., Chemistry of potential chromophoric groups in beech lignin, *Tappi*, **56**(5), 1973, 124–126.
63. Nimz H., Beech lignin – Proposal of a constitutional scheme, *Angew. Chem.*, **13**, 1974, 313–321.
64. Lundquist K., Proton (^{1}H) NMR spectroscopy, in *Methods in Lignin Chemistry* (Eds. Lin S.Y. and Dence C.W.), Springer-Verlag, Berlin Heidelberg, Germany, 1992, pp. 242–249.
65. Robert D., Carbon-13 nuclear magnetic resonance spectrometry, in *Methods in Lignin Chemistry* (Eds. Lin S.Y. and Dence C.W.), Springer-Verlag, Berlin Heidelberg, Germany, 1992, pp. 250–273.
66. Ludemann H.-D., Nimz H., Carbon-13 nuclear magnetic resonance spectra of lignins, *Biochem. Biophys. Res. Comm.*, **52**, 1973, 1162–1169.
67. Nimz H.H., Robert D., Faix O., Nemr M., Carbon-13 NMR spectra of lignins. 8. Structural differences between lignins of hardwoods, softwoods, grasses and compression wood, *Holzforschung*, **35**, 1981, 16–26.
68. Lundquist K., NMR studies of lignins. 2. Interpretation of the ^{1}H NMR spectrum of acetylated birch lignin, *Acta Chem. Scand.*, **B33**, 1979, 27–30.
69. Lundquist K., Paterson A., Ramsey L., NMR studies of lignins. 6. Interpretation of the ^{1}H NMR spectrum of acetylated spruce lignin in a deuterioacetone solution, *Acta Chem. Scand.*, **B37**, 1983, 734–736.
70. Bardet M., Foray M.-F., Robert D., Use of the DEPT pulse sequence to facilitate the ^{13}C NMR structural analysis of lignins, *Macromol. Chem.*, **186**, 1985, 1495–1504.
71. Gellerstedt G., Robert D., Quantitative ^{13}C NMR analysis of kraft lignins, *Acta Chem. Scand.*, **B41**, 1987, 541–546.
72. Robert D., Chen C.-L., Biodegradation of lignin in spruce wood by *Phanerochaete chrysosporium*: Quantitative analysis of biodegraded spruce lignins by ^{13}C NMR spectroscopy, *Holzforschung*, **43**, 1989, 323–332.
73. Zhang L. and Gellerstedt G. Quantitative 2D HSQC NMR determination of polymer structures by selecting suitable internal standard references. *Magn. Res. Chem.* **45**, 2007, 37–45.
74. Capanema E.A., Balakshin M.Y., Kadla J.F., A comprehensive approach for quantitative lignin characterization by NMR spectroscopy, *J. Agr. Food Chem.*, **52**, 2004, 1850–1860.
75. Gellerstedt G., Lindfors E.-L., Lapierre C., Monties B., Structural changes in lignin during kraft cooking. Part 2. Characterization by acidolysis, *Svensk Papperstidn*, **87**, 1984, R61–R67.
76. Lapierre C., Pollet B., Monties B., Rolando C., Thioacidolysis of spruce lignin: GC–MS analysis of the main dimers recovered after Raney nickel desulphuration, *Holzforschung*, **45**, 1991, 61–68.
77. Sjöström E., *Wood Chemistry. Fundamentals and Applications*, 2nd Edition, Academic Press Inc., San Diego, USA, 1993. p. 82.
78. Saka S., Goring D.A.I., The distribution of lignin in white birch wood as determined by TEM–EDXA, *Holzforschung*, **42**, 1988, 149–153.
79. Grunwald C., Ruel K., Kim Y.S., Schmitt U., On the cytochemistry of cell wall formation in poplar trees, *Plant biol.*, **4**, 2002, 13–21.
80. Evtuguin D.V., Amado F.M.L., Application of electrospray ionization mass spectrometry to the elucidation of the primary structure of lignin, *Macromol. Biosci.*, **3**, 2003, 339–343.
81. Lawoko M., Henriksson G., Gellerstedt G., New method for the quantitative preparation of lignin–carbohydrate complex from unbleached softwood kraft pulp: Lignin–polysaccharide networks I., *Holzforschung*, **57**, 2003, 69–74.
82. Lawoko M., Berggren R., Berthold F., Henriksson G., Gellerstedt G., Changes in the lignin carbohydrate complex in softwood during kraft and oxygen delignification: Lignin–polysaccharide networks II., *Holzforschung*, **58**, 2004, 603–610.
83. Lawoko M., Henriksson G., Gellerstedt G., Characterization of lignin–carbohydrate complexes from spruce sulfite pulp, *Holzforschung*, **60**, 2006, 162–165.
84. Gellerstedt G. and Zhang L. Chemistry of TCF-bleaching with oxygen and hydrogen peroxide, in *Oxidative Delignification Chemistry. Fundamentals and Catalysis*, Ed. Argyropoulos D.S., *ACS Symposium Series* **785**, 2001, pp. 61–72.
85. Goring D.A.I., Polymer properties of lignin and lignin derivatives, in *Lignins. Occurrence, Formation, Structure and Reactions* (Eds. Sarkanen K.V. and Ludwig C.H.), Wiley-Interscience, New York, USA, 1971, pp. 695–768.
86. Gierer J., Lindeberg O., Reactions of lignin during sulfate pulping. Part XIX. Isolation and identification of new dimers from a spent sulfate liquor, *Acta Chem. Scand.*, **B34**, 1980, 161–170.
87. Niemelä, K. "*Low-Molecular-Weight Organic Compounds in Birch Kraft Black Liquor*". Ph.D. Thesis, Helsinki University of Technology, 1990.
88. Mörck R., Yoshida H., Kringstad K., Hatakeyama H., Fractionation of kraft lignin by successive extraction with organic solvents. I. Functional groups, ^{13}C NMR-spectra and molecular weight distributions, *Holzforschung*, **40**(Suppl.), 1986, 51–60.
89. Mörck R., Reiman A., Kringstad K., Fractionation of kraft lignin by successive extraction with organic solvents. III. Fractionation of kraft lignin from birch, *Holzforschung*, **42**, 1988, 111–116.
90. Gellerstedt G., Majtnerova A., Zhang L., Towards a new concept of lignin condensation in kraft pulping. Initial results, *C. R. Biol.*, **327**, 2004, 817–826.

91. Gellerstedt G., Lindfors E.-L., Structural changes in lignin during kraft pulping, *Holzforschung*, **38**, 1984, 151–158.
92. Lin S.Y. and Detroit W.J. Chemical heterogeneity of technical lignins – Its significance in lignin utilization. *1st International Symposium on Wood and Pulping Chemistry*, Stockholm, Sweden, 1981, Proceedings, Volume 4, pp. 44–52.
93. Hassi H.Y., Chen C-L. and Gratzl J.S. Carbon-13 NMR spectroscopic characterization of lignosulfonates. *Tappi Research and Development Conference*, Appleton, USA 1984, Proceedings, pp. 249–260.
94. DeLong E.A. Method of rendering lignin separable from cellulose and hemicellulose in lignocellulosic material and the product so obtained. Can. Pat. #1,096,374, 1981.
95. Josefsson T., Lennholm H., Gellerstedt G., Steam explosion of aspen wood. Characterization of reaction products, *Holzforschung*, **56**, 2002, 289–297.
96. Robert D., Bardet M., Lapierre C., Gellerstedt G., Structural changes in aspen lignin during steam explosion treatment, *Cell. Chem. Technol.*, **22**, 1988, 221–230.