

1 Preparation and Characterization of Lignin-Carbohydrate Complexes

To explain the difficulty in separating lignin from carbohydrates in wood, Erdman (1866) hypothesized that the two combined chemically to form “glycolignose”. This hypothesis was accepted by many in his day. Since that time, numerous investigations have been made on the nature of the association between lignin and carbohydrates in wood.

Merewether (1957) published an excellent review of the phenomena conjectured to arise from lignin-carbohydrate complexes (LCCs) in the course of the chemical or microbiological treatment of wood. Of particular interest in that review are the references to the research of Traynard et al. (1953), who had already extracted a water-soluble LCC from poplar wood.

1.1 Extraction with Hot Water or a Dilute Alkaline Solution

1.1.1 Early Research into Water-Soluble Lignin-Carbohydrate Complexes

Traynard et al. (1953) had isolated water-soluble LCCs from poplar wood using hot water (140°C). The yield was 16–18% of the original wood. After acetone was added to the aqueous extract until it contained 96% acetone, the precipitated polysaccharides were removed by filtration, leaving the LCC in solution. The LCC contained 9.7% methoxy and 50.5% carbon and gave reducing sugars on hydrolysis with mineral acid, together with an insoluble lignin containing 17.5% methoxy and 63% carbon. Traynard was the first to describe the extraction of LCCs, and his report was followed by numerous others. Merewether (1954) verified the existence of a water-soluble lignin in an aqueous mother liquor on ethanolysis of eucalyptus wood. Bolker and Wang (1969) isolated a water-soluble lignin-xylan complex with hot water and the Björkman LCC (Sect. 1.2.1) with dimethylsulfoxide from an extractive-free milled white birch wood. On acid hydrolysis, both of the LCCs released insoluble lignin and soluble carbohydrates in an aqueous solution. The Klason lignin content was 11.3% in the Björkman LCC and 4.2% in the lignin-xylan complex. Based on the result, Bolker and Wang proposed the existence of a covalent bond between lignin and carbohydrates (Table 1.1). Both LCCs contained 0.2% amide-type nitrogen after ammonolysis, and hence were expected to contain an equal

Table 1.1. Properties of the lignin-carbohydrate complexes. (Bolker and Wang 1969)

	LXC	LCC ^a	LCC ^b
Duration of ball-milling (h)	30	48	48
Yield (%) of oven-dry wood	22.1	15.6	15.0
Klason lignin (%)	4.2	11.3	13.0
Uronic acid (%)	15.2	16.3	16.7
Sugars (relative amounts)			
Xylose	75	82	86
Galactose	3	3	9
Glucose	7	8	9
Mannose	10	6	3
Arabinose	5	1	2

^a Prepared from white birch (*Betula papyrifera*) according to Björkman.

^b Results of Björkman for silver birch (*Betula verrucosa*).

amount of glucuronic acid ester. However, there was no evidence for ester linkages between the uronic acid and lignin, unlike for the esters of uronic acid carboxyls with hydroxyls of xylose units on the same or other xylan chains. Similarly, water-soluble lignin-hemicellulose complexes were isolated by Kringstad and Cheng (1969b) from spruce chlorite holocellulose. Three specimens were prepared, a lignin-hemicellulose mixture from milled wood lignin, a lignin-hemicellulose complex isolated with polysaccharidase, and the native lignin-hemicellulose complex; these were compared with the aid of gel-filtration diagrams. The lignin and hemicellulose components of the native complex were of the same molecular weight range. Kringstad and Cheng concluded that the lignin was chemically combined with the polysaccharides. Thus, water-soluble lignin (that is, the LCC) had already been recognized to exist, though no direct evidence had been obtained.

Merewether and Samsuzzaman (1972) used milled wood of *Eucalyptus obliqua* passed through a 0.75-mm screen and extracted the water-soluble LCC from the wood flour after pre-extracting a kino-type resin by boiling in a 0.5% sodium hydroxide solution (Fig. 1.1). The pre-extracted milled wood (5.97 kg) was extracted with a boiling 0.5% sodium hydroxide solution for 6 h, the pH of the alkaline solution was lowered to 2.5 with sulfuric acid, and the precipitated alkali lignin (125 g) was removed. The filtrate was made alkaline, a crystalline sodium sulfate was removed, and the solution was acidified again, after which a benzene extract (10 g) and hemicelluloses (29 g) were recovered. To the dried supernatant, acetone was added, and the insoluble matter removed after recovery of the soluble component (lignin 58 g). The acetone-insoluble portion was divided into six fractions on a column of cellulose after removal of the water-insoluble matter by filtration. The first and the second fractions were identified as

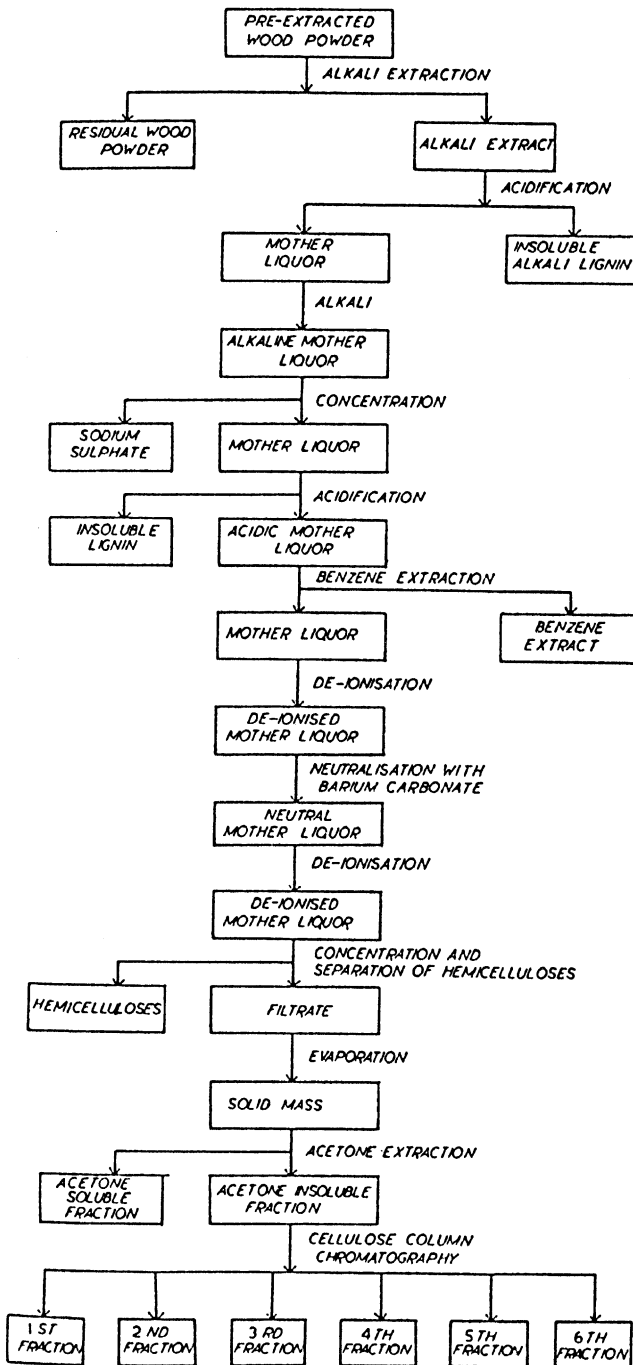


Fig. 1.1. Isolation of a lignin-carbohydrate complex. (Merewether and Samsuzzaman 1972)

water-soluble LCCs in that they contained 19.1% Klason lignin, 33% carbohydrates, and 4.4% ash. Gas-liquid partition chromatography (GLC) and paper chromatography analyses showed that the carbohydrates consisted of xylose, arabinose, galactose and uronic acid residues. Merewether et al. (1972) studied the LCC in more detail by proton NMR spectroscopy. They prepared six specimens, namely, acetylated LCC(I), the diazomethane-methylated product of acetylated LCC(II), the diazomethane-methylated LCC(III), the acetylated product of diazomethane-methylated LCC(IV), the acetylated product of acid-hydrolyzed LCC(V) and the diazomethane-methylated product of acid-hydrolyzed LCC(VI). NMR analyses of these specimens indicated the presence of phenolic OH $0.6/C_9$ and total uronic acids $0.5/C_9$ (25.5%), and the free carboxyl group originating from the lignin component of the LCC was thus calculated to be $0.1/C_9$. However, the result was ambiguous because of overlapping NMR signals. According to Adler (1961), the intensity of the α -vinyl proton in the phenylpropane unit should increase on acid hydrolysis of the sugar components of the LCC if sugar chains combine with the α -carbon of the phenylpropane side chains of the lignin. An overall increase of intensity was observed in this region in NMR spectra of the acetylated LCC hydrolysate, though the quantitative determination was incomplete.

Kringstad and Ellefsen (1964) and Kringstad (1965) delignified spruce wood using chlorite and fractionated the extracted water-soluble polysaccharides by a gel-filtration technique. They showed that the lignin and polysaccharide contained in the water-soluble fraction had the same range of molecular weights as on the chromatogram. They also prepared a partly delignified spruce wood using chlorine-monoethanolamine and isolated a water-soluble glucomannan-lignin complex containing 12% lignin as well as a glucuronoxylan-lignin complex with 4% lignin.

1.1.2 Extraction with Hot Water of Finely Divided Wood

As will be described in Section 1.2.1, Björkman's extraction method, first reported in 1957, has been used widely but has the disadvantage of requiring solvents with a high boiling point, such as dimethylformamide or dimethylsulfoxide, for extracting the LCCs. Furthermore, this method is unsuitable for large-scale extraction of LCCs from wood. Watanabe et al. (1987) proposed a more convenient method of extracting LCCs with cold and hot water after treatment with 80% aqueous dioxane of a finely divided wood flour less than 35 μm in diameter. The LCC obtained by this method, outlined, in Fig. 1.2, was named LCC-WE, and compared with the Björkman LCC. The yield of LCC-WE in extractive-free red pine (*Pinus densiflora*) wood was 9.3%, a value in between the 12.8% for the Björkman LCC extracted twice with dimethylsulfoxide (Koshijima et al. 1976) and the 5.31% for the Björkman LCC extracted twice with dimethyl-

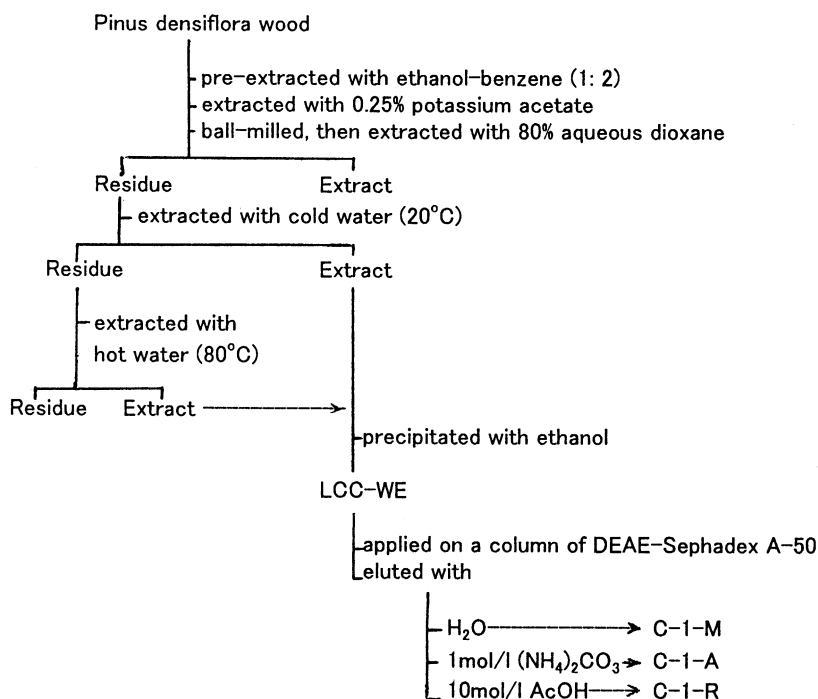


Fig. 1.2. Isolation of the water-soluble lignin-carbohydrate complex (LCC-WE) from *Pinus densiflora* wood. (Watanabe et al. 1987)

formamide (Koshijima et al. 1976). Yields of the three subfractions of the LCC-WE mentioned in Section 1.3.1 are 43.1% (C-1-M), 48.7% (C-1-A), and 2.1% (C-1-R), respectively (see Table 1.2). For the Björkman LCC, the yields of the corresponding subfractions were 50–55, 24–26 and 3.5–4.3%, respectively. The two-fold difference in C-1-A should be noted. The lignin content of this fraction was 26.6%, about double that of the C-1-A in the Björkman LCC, implying that this method is more effective for extracting the acidic component of the LCCs. As shown in Tables 1.2–1.4, the chemical composition of the neutral subfraction, C-1-M, of LCC-WE is Man:Glc:Gal=3.4:1:0.2, acetyl 7.6% and $[\alpha]_D-28.2$, indicating that the main component is an acetyl glucomannan as with the Björkman LCC, and that all of the galactose is located at the non-reducing end of the polysaccharide chains, including single side chains (Table 1.5; Sect. 1.2.1). Table 1.3 shows that the carbohydrate moiety of C-1-A is composed of glucomannan and arabinoglucuronoxylan, mostly the latter.

Of note is that an appreciable amount of arabinose and galactose residues occur at the non-reducing end in the respective polysaccharides, as is shown in Table 1.5. The results imply that the polysaccharide chains in subfraction C-1-A consist of the above two polysaccharides, both of which

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and Other Plant Tissues

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