

CHARACTERIZATION OF OLD WOOD SAMPLE FROM LAKE ERIE

FINAL PROJECT REPORT, PROJECT 101-99

Alison L. Spongberg, Department of Geology, University of Toledo, Toledo, Ohio 43606-3390
aspongb@utnet.utoledo.edu

Abstract

Samples of wood aging from 0-8200 years before present were dated and analyzed to characterize their lignin content. Samples were obtained from the Great Lakes region, with the oldest samples being recovered from sediments in Lake Michigan. All samples were either white oak (angiosperm) or spruce/pine (gymnosperm) woods. Lignins were quantified as acid soluble and insoluble fractions. Nitrobenzene oxidation products were obtained to determine the major chemical components of the lignin. Total lignin content increased with increasing age of the samples due to the loss of the less recalcitrant plant components (cellulose, hemicellulose). Plant order (gymnosperm versus angiosperm) could be discerned by the distribution of phenyl propane units isolated in the nitrobenzene oxidation products. Fortunately, this characteristic was preserved even in the oldest wood samples, with no indication that the lignin were degrading at all. This technique will now be used for two future projects (1) Sediment cores from Lake Erie will be analyzed to help identify climatic changes within the past 10,000 years, and (2) Dated marsh sediment cores will be analyzed and correlated with pollen data to help identify the onset of deforestation and agriculture in the 19th Century.

Background Information

The three most important cell wall constituents in most plants are cellulose, hemicellulose and lignin. Cellulose is a polymer of glucose molecules. The simplicity of the cellulose structure means that the enzymes required for degradation are small and therefore, degradation is rapid. Hemicelluloses cross-link between cellulose and lignin by covalent bonds, most importantly, ether bonds. Hemicelluloses are branched polymers of xylose, arabinose, galactose, mannose, and glucose (Richard, 1996). This matrix of lignin and hemicellulose enhances plant cell wall stability while challenging microbial degradation.

Lignins have widespread occurrence and are geochemically significant. In plants, lignins are synthesized by dehydration and condensation of aromatic alcohols such as coniferyl, sinapyl

and coumaryl alcohols. By identifying the major differences in lignins from different plant types, lignin can be used as a fingerprint or biomarker of the sediments in which it is contained. The chemical structure is characterized by aromatic phenolic units, which are not usually synthesized by animals. The complete structure of lignin has a molecular weight around 10,000amu (Figure 1). The polymer is quite resistant to chemical degradation and almost impervious to enzymatic digestion. Lignins are perhaps more resistant to enzymatic degradation than any other naturally occurring polymer. Although under certain conditions white rot fungus can degrade lignin our preliminary studies indicate that in the Great Lakes sedimentary environment, lignins and other fossil plant compounds persist.

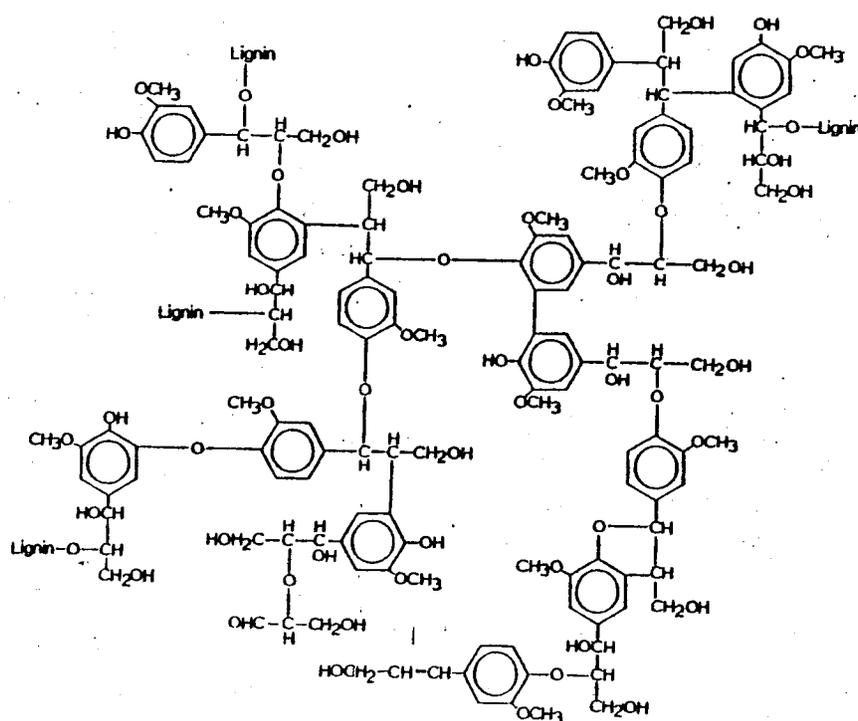


Figure 1: Structure of lignin

Terrestrial higher plants are the fourth major source of organic matter in sediments, largely due to the presence of lignin. Lignin is not found in marine or lower plants such as algae, fungi, and mosses. By identifying the major differences in lignins from different plant types, lignin can be used as a fingerprint or biomarker of the sediments in which it is contained. Major differences in lignins from gymnosperms (which predominate in colder climates), angiosperms (which

lignin oxidation products from deciduous and coniferous trees enable a close study of degradation of lignin with age and environment (aerobic versus anaerobic).

Lignin is depolymerized extensively and is more soluble in alkaline than under acidic or neutral conditions. Alkaline conditions also preserve the aromatic ring structures. The use of nitrobenzene or cupric oxide as catalysts cause cleavage at ether bonds giving a mixture of aromatic aldehydes, ketones, and acids. The recalcitrant polysaccharide can be used as a unique fingerprint of both deciduous and coniferous trees.

Previous Work

Much of the research on lignin has been produced by the pulping industry regarding the removal of lignin to improve paper quality. Mechanical pulping does not remove lignin, therefore chemical pulping methods used in the manufacture of paper are designed to remove the lignin by conversion to water-soluble products. This is usually accomplished by cooking wood chips with acidic sulfite solutions or with alkaline sulfate-sulfide solutions. Lignin is further removed or modified through bleaching steps to give wood pulp greater brightness. The extracted lignin is mainly a waste product, which is burned or discharged into streams.

Lignin biodegradation is very important in maintaining nature's carbon cycle. Lignin degradation is primarily an aerobic process, and in anaerobic environments lignin can persist for very long periods (Hammel 1997). Since lignin is interconnected by stable ether and carbon-carbon bonds, decomposition mechanisms must be oxidative rather than hydrolytic. Warm temperature, high moisture content, available oxygen, and high palatability of plant litter to microorganisms all favor decomposition. More highly lignified tree litter requires more specialized organisms for degradation.

In wood, three distinct types of aerobic fungal decay can be distinguished: white rot, brown rot, and soft rot (Eriksson et al., 1990). White rot fungi are the most abundant degraders of wood in nature. They decompose the lignin in wood to gain access to the cellulose and hemicellulose that are embedded in the lignin matrix. White rot fungi are able to decompose lignin under optimal conditions at rates similar to polysaccharide degradation (oxidation) (Hammel 1997). Brown and soft rot fungi are less important degraders in a quantitative sense. They attack the cellulose and hemicellulose components of trees while circumventing the lignin. Aerobic fungal degradation of lignin results in increased acidity and methoxyl demethylation (Crawford, 1981) by attacking the polysaccharide component (Hammel 1997) with the eventual loss of the characteristic lignin signature.

Table 1: Samples used in the Lignin Study. C designations are gymnosperms, D designations are angiosperms.

Sample ID	Wood Type and Age	Site Location
0-C	Conifer, Fresh	Toledo, Ohio
2-C	Conifer, 2 years	Toledo, Ohio
8200-C1	Spruce stump, 8200 years	Jacksonport, Michigan
8200-C2	Spruce stump, 8200 years	Jacksonport, Michigan
8200-C3	Spruce stump, 8200 years	Jacksonport, Michigan
8200-C4	White cedar (small log), 8200 years	Jacksonport, Michigan
0-D	White Oak, Fresh	Toledo, Ohio
2-D	White Oak, 2 years	Toledo, Ohio
400-D	White Oak stump, 400 years	Tiffin, Ohio
8200-D1	White Oak limb, 8200 years	Olson site, Michigan
8200-D2	White Oak limb, 8200 years	Olson site, Michigan

Samples previously analyzed and included in this report for completeness were collected in Toledo, Ohio during the spring of 1997. Coniferous (*Pinus*) and deciduous (*Quercus*) wood samples of 0 and 2 year old wood were obtained from fresh cuttings and compost piles. These samples are designated 0-D, 2-D, 0-C, and 2-C for age and tree order, respectively.

Samples from the remains of forest on the floor of southern Lake Michigan were made available for 'very old' wood. The Olson site is located offshore Illinois in southern L. Michigan approximately 25 km east of the lighthouse at the entrance to the Chicago Harbor (41° 49'N, 87° 18'W) (Chrzastowski et al., 1991; Pranschke, 1993). This site is interpreted as the remains of a wetland forest that was established during the post-glacial, extreme low-lake phase (Chippewa low) and then inundated during lake level rise (Nipissing transgression) (Pranschke and Shabica, 1993). The mean age of this forest is 8,200 years BP according to radiometric dating (Pranschke and Shabica, 1993).

A variety of sizes and shapes occurred among the stumps. For all exposed parts of the stumps, no bark is preserved. The degree of surface abrasion, combined with evidence for local sediment transport, suggest that the stumps may have been periodically exposed and buried during

Gas Chromatographic Method

One microliter of sample was injected into a Hewlett Packard 6890 gas chromatograph with DB-5 fused silica capillary column (30m x 0.32mm i.d., film thickness 0.25 μ m). Helium was used as the carrier gas at a flow rate of 2ml/min. Injection temperature was 200°C. The oven temperature was programmed at 60°C for one minute, then ramped at 10°C/ min to 250°C for a total run time of 30 minutes. Helium was the carrier gas at a flow of 0.9 ml/min. The mass selective detector was run in Scan mode with scan range of 45.0-450.0 amu, sample rate of 2 and threshold of 1000.

It should be noted that these methods are partly empirical, therefore slightly different results may occur under different experimental conditions.

Results and Discussion

Total Lignin

Extraction data and percent acid soluble insoluble and total percent lignin are presented in Figure 2. These data are ordered by age. Note that the acid soluble percent is multiplied by a factor of ten. These data are averages for three different samples each.

As expected the amount of extractable organic matter is larger in the younger wood samples. With time, these compounds are either leached from the samples or degraded. Nevertheless, all wood samples contained an adequate amount of organic extract for subsequent analyses. Percent of acid soluble lignin was also higher in the younger wood samples. Presumably this fraction is also easily lost by leaching or degradation. The acid insoluble lignin is about twice as high a percent of the total wood in the older tree samples as in the younger samples. As much as 70% of the wood samples is insoluble lignin in the 8000 year samples, due to the degradation of the cellulose and hemicellulose tissues. Interestingly, the 400 year sample contains the highest amount of acid soluble lignin and still retains a large percentage of non- lignin tissue. In general, the 400 year oak sample remains much like the young 0-2 year wood samples. Furthermore, no difference between tree types is evident in these data.

Oxidation Products

Nitrobenzene oxidation of the extracted wood meal produced the oxidation products shown in Figure 3. These data are arranged by wood type. Interestingly, the oxidation products are still evident in the oldest wood samples, with some of the oldest samples showing the

largest absolute quantities of the compounds. The coniferous samples show only vanillin, with increasing amounts with increasing age. This correlates to the increase in insoluble lignin. Syringaldehyde is evident only in the deciduous samples, whereas acetosyringone is evident in the deciduous samples, excluding the 400 year sample. The amounts of these compounds even in the 8200 year samples are still appreciable and easily detected.

The youngest samples (0 and 2 years) were obtained from wood in a relatively dry oxidizing surface environment. The 400 year sample was extracted from a dense till layer in the portion of the soil profile where the water table fluctuates. This results in anaerobic to potentially aerobic conditions which change seasonally. The 8200 year samples were in anaerobic lake bed sediments, with unknown but possible periods of exposure to oxidizing conditions. Despite these environmental differences, all samples retained the lignin signatures that are diagnostic to the wood type. With the limited number of samples so far analyzed, a statistically significant trend of lignin composition with age cannot be obtained. However, the data are intriguing and suggest that useful lignin is retained in wood as old as the Lake Erie sedimentary record to the last major glaciation. It is known that lignin persists in aerobic environments, but it appears from these data that the fluctuating vadose zone has also remained sub-optimal for degradation for at least 400 years, if not longer.

Future Studies

From this study many avenues of research are being explored. The applications are:

- ✓ Studies are being conducted to determine the feasibility of using lignin as climatic biomarkers for Lake Erie basin sediments.
- ✓ Extraction of lignin from sediments is currently being attempted. If successful this lignin will be used to examine marsh sediment cores in correlation with palynology to determine timing of deforestation around the marshes.
- ✓ Lignin in broad-leaf tropical trees of Central America will be used to trace the rates and effects of deforestation.

References cited

- Bonner, John, J.E. Varner, eds., 1965. *Plant Biochemistry*, Academic Press, New York.
- Chen, Chen-Loung, 1988. Characterization of lignin by oxidative degradation: Use of gas chromatography-mass spectrometry technique. *Methods in Enzymology* 161: 110-136.