Characterization of Southern Yellow Pine Bark Layers by Attenuated Total Reflectance (ATR) and Fourier Transform Infrared (FT-IR) Spectroscopy

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Abstract

The outer bark (rhytidome) of the southern yellow pines is a complex structure comprised of alternating layers of obliterated phloem and periderm tissues, with the latter comprised of three layers, those being phellem, phellogen, and phelloderm. An attenuated total reflectance (ATR) sampling accessory, coupled with a Fourier transform infrared (FT-IR) spectrometer, provided a facile means to probe the chemical nature of these layers to near nano-scale levels. Comparison of the spectra for both surfaces of the obliterated phloem specimens showed no significant differences whereas comparison of the spectra for the inner (phelloderm) and outer (phellem) surfaces of the periderm specimens were quite different. Relative to the spectrum for the outer periderm surface, the inner periderm surface had significantly greater signals for aliphatic and carbonyl functionalities. Spectral subtractions after solvent extractions were consistent with the removal of resinous and phenolic extractives. Results provide not only the first demonstration of the chemical functionalities of individual layer/surfaces in pine bark by FT-IR spectroscopy, but also new evidence suggesting moisture barrier properties for the phelloderm.

Introduction

The outer bark (rhytidome) of the southern yellow pines is a complex structure comprised of alternating layers of periderm and obliterated phloem tissues. As a tree grows, older inner bark (phloem) tissue is periodically sealed off by periderm formation. Outside of these newly-formed periderms, the sieve cells collapse and the vertical parenchyma expand through a process called obliteration, thus the phloem is transformed into obliterated phloem. Periderm formation is initiated by the development of phellogen (cork cambium) from parenchyma cells in the phloem. This meristematic layer of cells produces a phelloderm layer to the interior and phellem layer to the exterior. Together, these three layers, phelloderm, phellogen, and phellem, constitute a periderm. A drawing by Howard (Howard, 1971) shows the intricate architecture of a southern yellow pine periderm (Fig. 1).

An accepted function of the periderm is as a barrier to separate the non-living outer bark from the living inner bark (Howard, 1971; Martin, 1969; Martin and Crist, 1970).

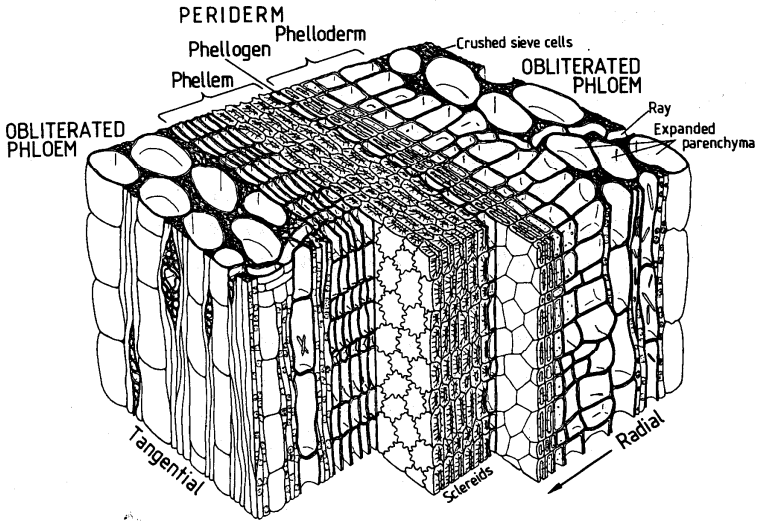

Fig. 1. Periderm and obliterated phloem tissues in southern yellow pine outer bark (Howard, 1971).

In an effort to develop value added products from bark, research in our laboratory has focused on the development of techniques to separate periderm and obliterated phloem tissues. Relative to the periderm, the obliterated phloem is low in density and is spongy in nature. Through a specific grinding process, a bimodal distribution of particles can be obtained with the coarser particles being rich in periderm, and the finer particles rich in fragments of obliterated phloem (Eberhardt and Reed, 2005; Eberhardt and Reed, 2007). Further processing of the periderm-rich fraction has afforded a plywood adhesive mix filler with superior performance over fillers prepared with whole bark, and especially that from the fraction rich in obliterated phloem (Eberhardt and Reed, 2005; Eberhardt and Reed, 2007).

Ongoing research involves both the development of quality control tools for the selection of preferred bark supplies and further characterizations of the bark to better understand how to best utilize this biomass resource. Recent studies have shown the utility of near infrared spectroscopy, coupled with partial least squares analysis, to determine the ratios of inner to outer bark in available bark resources (So and Eberhardt, 2006). With respect to bark characterization, the current study involves the dissection of bark into its components (periderm and obliterated phloem) and analysis of those components by (FT-IR) spectroscopy. At this juncture it should be noted that detailed studies on the anatomy of southern yellow pine bark have been published (Howard, 1971; Martin, 1969; Martin and Crist, 1970; Chang, 1954). However, innovations in FT-IR spectroscopy now provide the opportunity to probe the chemical nature of these anatomical features. Specifically, attenuated total reflectance (ATR) accessories are now available that provide a facile means to collect FT-IR spectra from small particles and surfaces in contact with a thin diamond

covering a crystal with a high refractive index (e.g., zinc selenide) (Himmelsbach et al, 2006). The ability to detect surface modifications as thin as 10 nm, such as that obtained with the treatment of wood with plasmas (Denes and Young, 1999), suggests that there is the potential to characterize bark, and other woody substrates, at nano-scale levels.

Materials and Methods

Bark collection and sectioning

Freshly peeled southern yellow pine (mostly Pinus taeda L.) bark was obtained at a local plywood plant; bark was removed from logs by a ring debarking system. Large strips of bark, with both outer and inner bark layers together, were allowed to dry under ambient conditions before cutting into small sections (ca. 1 cm^2). Bark sections were then carefully sliced with a razor blade to obtain thin slices of both periderm and obliterated phloem tissues. Specific attention was given to the periderm slices to assign surfaces to either being inside or outside the phellogen. Obliterated phloem layers were thick enough that clean slices, without adhering periderm, could be prepared and analyzed directly. Periderm layers were very thin and slices typically had a thin layer of obliterated phloem tissue on each surface that was removed by gently scraping with a razor blade.

FT-IR spectroscopy

FT-IR spectra were collected using a Thermo Nicolet Nexus 670 spectrometer equipped with a Thermo Nicolet Smart Golden Gate MKII Single reflection ATR accessory. Specimens were placed on the diamond crystal and pressure applied with the anvil affixed to the accessory clamping mechanism. A precision torque wrench was used to apply the same torque, 50 cNm, to the limiter screw on the accessory thereby achieving the same applied pressure for all samples. Three spectra collected per surface were averaged for 4 periderm and 4 obliterated phloem specimens. Hexane-extracted specimens were prepared by steeping in hexane (24 hr, RT) and then drying several days under ambient conditions before spectroscopic analysis. Additional specimens were extracted in an analogous manner with both hexane and then ethanol. Spectral subtractions were processed using the subtraction feature in the Omnic operating software (version 5.2) for the instrument.

Results and Discussion

During the dissection of the bark blocks, it was relatively easy to obtain seemingly homogeneous obliterated phloem specimens; examination of both surfaces showed no readily apparent difference in appearance. For the periderm specimens, scraping with a razor blade was necessary to remove adhering obliterated phloem tissue. During this process, it was observed that the outer surface had a smooth brown appearance whereas the inner surface often had what appeared to be a yellowish deposit. Observations under a dissecting microscope showed that the thin-walled cork cells were mostly removed from the outer surface thus exposing the stone cells (sclereids) of the phellem. The inner surface appeared to retain some of the phelloderm and thus also phellogen.

Averaged FT-IR spectra for the obliterated phloem and periderm specimens are shown in Fig. 2. Comparison of the spectra for both surfaces of the obliterated phloem specimens showed no apparent differences, and so, only one representative spectrum is shown. For the periderm specimens, comparison of the spectra for the outer and inner surfaces demonstrated different chemical functionalities. Relative to the spectrum for the obliterated phloem, both periderm spectra had significantly greater signals for aliphatic C-H (2918, 2850 and 1463 cm^{-1}) and carbonyl (1737 cm^{-1}) functionalities typically present in resin and fatty acids as well as the polymeric plant waxes. The presence of such hydrophobic materials confirms the suggested function of periderm as a moisture barrier. Aromatic signals between 1634-1598 (C=C stretch) and 1511 cm^{-1} (C-C vibration) were also significantly greater in the periderm spectra and indicate the presence of phenolic extractives and/or heavily lignified cell walls. The above-mentioned aliphatic, carbonyl and aromatic signals could also be attributed to the presence of suberin, a polymeric plant substance with aliphatic and aromatic domains that generally serves as a sealant to impede desiccation and as a barrier to microorganisms, especially in periderms formed in response to wounding (Bernards, 2002).

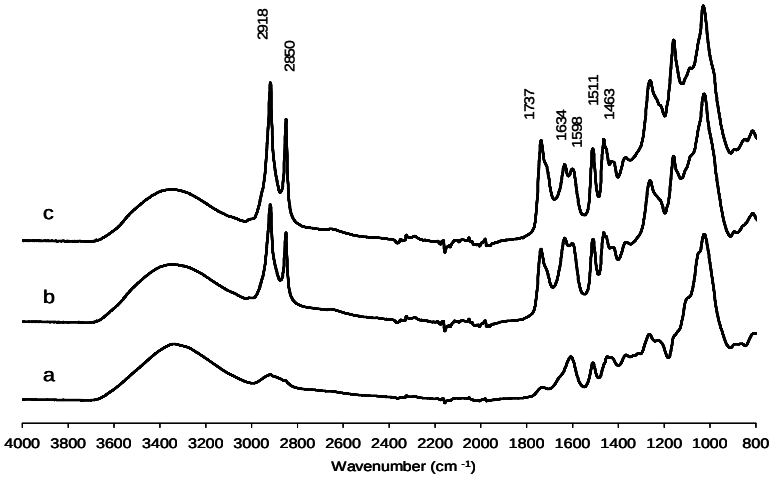

Fig. 2. FT-IR spectra: obliterated phloem (a), outer (phellem) surface (b) and inner (phelloderm) surface (c) of periderm.

Comparison of the spectra from the inner and outer periderm surfaces showed higher relative signal intensity for aliphatic and carbonyl functionalities on the inner surface (phelloderm side). Thus, relative to the stone cells in the phellem, the phelloderm would appear to have higher a content of hydrophobic substances. Specimens were extracted with hexane, dried and analyzed again to see if this difference between the two surfaces could be attributed to a greater presence of non-polar extractives (e.g., resin and fatty acids). The average spectra for the extracted specimens were subtracted from the corresponding average spectra from the unextracted specimens to obtain the difference spectra in Fig. 3 as an indirect assessment of the functionalities of any extractives removed. For the inner surface, a significant amount of resin and fatty acids were likely removed as evidenced by signals consistent with these extractives (aliphatic C-H (2917, 2849 cm^{-1}) and carbonyl

(1737, 1712 cm^{-1})). For the outer surface, a significant amount of phenolic extractives were likely removed as evidenced by the high relative signal intensities (1631-1599, 1511 cm^{-1}) consistent with aromatic substances. Together, these results suggest a stratification of the extractives in a periderm. Moreover, in addition to the suberized cork cells in the phellem (Howard, 1971; Bernards, 2002), there is now evidence that the phelloderm may also impart moisture barrier properties to the periderm.

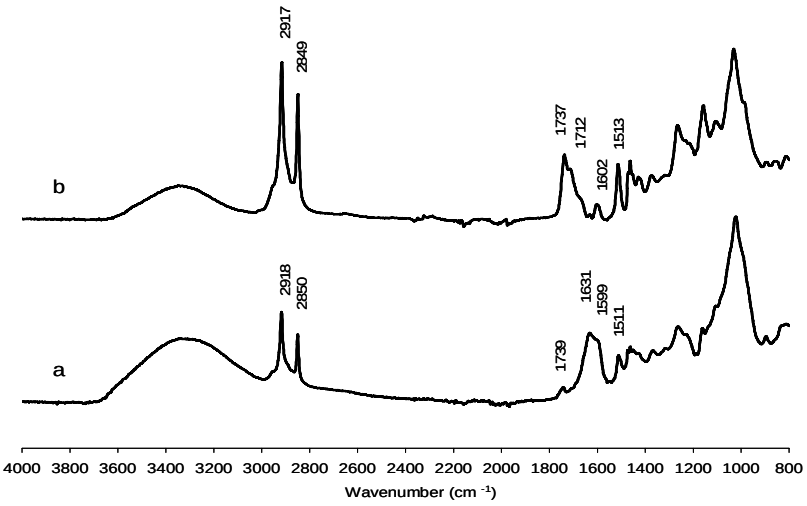

Fig. 3. FT-IR difference spectra of hexane-extracted specimens from unextracted specimens: outer (phellem) surface (a) and inner (phelloderm) surface (b) of periderm.

A preliminary assessment of the presence of polar extractives was provided by the extraction of hexane-extracted specimens with ethanol. Subtracting the average spectra from the ethanol-extracted specimens from the respective average spectra from hexane-extracted specimens afforded the difference spectra shown in Fig. 4.

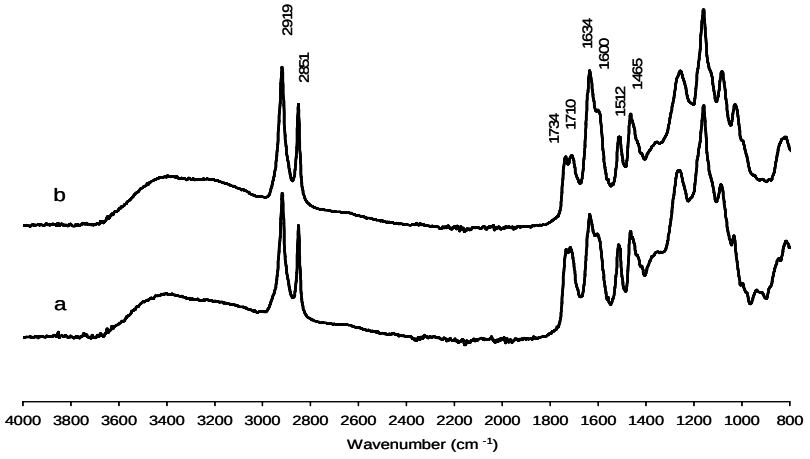

Fig. 4. FT-IR difference spectra of ethanol/hexane-extracted specimens from hexane-extracted specimens: outer (phellem) surface (a) and inner (phelloderm) surface (b) of periderm.

Results for the periderms suggested that similar materials removed from both the outer and inner surfaces; along with the above-mentioned signals attributed aliphatic and carbonyl functionalities, signals consistent with aromatics (1634-1600, 1512 cm^{-1}) were significantly greater. Along with signals attributable to hydroxyl functionalities (ca. 3300 cm^{-1}), this suggests the removal of phenolic extractives (lignans, condensed tannins).

Conclusions

Comparison of the spectra for both surfaces of the obliterated phloem specimens showed no significant differences whereas the inner (phelloderm) and outer (phellem) surfaces of the periderm specimens were quite different. The difference spectra after solvent extractions suggested the removal of resin and fatty acids from the inner surface and phenolic extractives from the outer surface. Thus, the extractives in these periderms appear to be stratified. In addition to the suberized cork cells of the phellem, hydrophobic substances in phelloderm may also impart moisture barrier properties to the periderm.

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