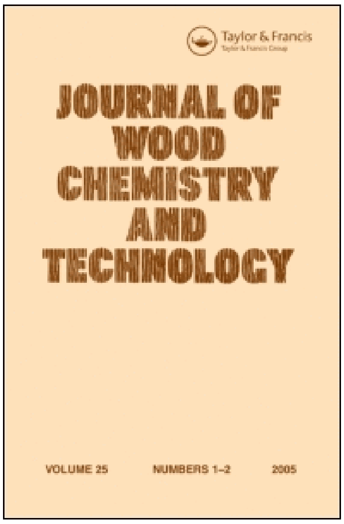

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DETECTION OF PINOSYLVINS IN SOLID WOOD OF SCOTS PINE USING FOURIER TRANSFORM RAMAN AND INFRARED SPECTROSCOPY

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ABSTRACT

Diffuse reflectance Fourier transform infrared (DRIFT) and near infrared (NIR) FT-Raman spectroscopy were used to detect pinosylvins in the wood of *Pinus sylvestris* L. trees. NIR FT-Raman spectroscopy offered the possibility of revealing pinosylvins simply by visual inspection of Raman spectra whereas DRIFT spectra needed a more complicated evaluation. Pinosylvin and resin acids from Scots pine were examined as to the possibility of their being the cause of observed spectral differences between sapwood and heartwood. Since pinosylvins are important compounds for the decay resistance of Scots pine wood, the detection of pinosylvins with Raman spectroscopy might be used to assess durability of wood products.

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INTRODUCTION

Scots pine (*Pinus sylvestris* L) is one of the most important timber trees in Europe. The heartwood has for centuries been utilized because of its high resistance to wood destroying fungi. The presence of high concentrations of resin acids in the heartwood promotes durability because they restrict the uptake of water.[1] Furthermore, the heartwood of Scots pine is characterized by the presence of pinosylvin, 3,5-dihydroxystilbene and its monomethyl ether, 3-hydroxy-5-methoxy stilbene.[2,3] These compounds have been shown to have a high antifungal activity and can not be found in the sapwood.[4,6] Pinosylvins are synthetizised in the transition zone between sapwood and heartwood when heartwood is formed.[4-6]

Since the amount of pinosylvins varies between individual trees within rather wide limits,[4] it should be possible to use the pinosylvin content as an indicator of decay resistance when selecting timber trees for special purposes. So far, GLC [7] or HPLC [8,9] are the preferred methods for the determination of pinosylvin, but these techniques require grinding and extraction of samples and are quite time-consuming. In wood anatomical research, a rapid method is also needed for studies of the relationship between biochemical and anatomical changes during heartwood formation, which occur within parts of an annual ring. The determination of pinosylvins by spectroscopic methods directly on the surface of a solid wood sample may therefore be an alternative method to GC and HPLC, especially since the fungal activity is expected to start at the surface of a wood sample. IR and Raman spectroscopy are two rapid, non-invasive and sensitive methods for the detection of chemical structures. For solids only the surface of the sample is probed, the penetration depth being only a few microns.

In the present study the two spectroscopic methods, NIR-Raman and IR, are compared with respect to their ability to differentiate the pinosylvin rich heartwood from the sapwood of Scots pine.

EXPERIMENTAL

Wood samples

Five uniform wood blocks (70x10x20 mm) were cut from each of two pine trees, 49 and 57 years old. The blocks were cut out from discs located at breast height. Discs from both trees showed a distinct boundary between heartwood and sapwood when stained with sulfanilic acid and nitrite. Prior to analysis the wooden blocks were sanded with a fine paper to smooth the surface. Spectra were recorded on the transverse surface of the wood block from the bark to the pith.

To verify the identity of pinosylvin with Raman-spectroscopy, one wood block was devided into two pieces (70x10x20 mm), one of which was impregnated with 5 mL of acetone and the other with 5 mL of pinosylvin standard dissolved in acetone (2% W/W) at normal atmospheric conditions and thereafter air dried. The spectra of the samples were measured immediately before the addition of the solutions and one and three days after the addition.

Raman analyses

Raman spectra were recorded using a Perkin Elmer PE1760X Fourier transform infrared spectrometer equipped with a near-infrared (NIR) Raman bench. Data storage and manipulation was carried out using the Perkin Elmer IR Data Manager *(IRDM)* software package. The Raman scattering was excited with an intensity-stabilized (0.1% r.m.s.) 1064 nm emission from a Spectron SL 301 neodymium-doped yttrium aluminium garnet *(Nd:YAG)* laser, and the scattered light collected with a 180° backscattering-geometry lens. The spectra presented are typically 100 accumulations at 4 cm^{-1} resolution, using an indium gallium arsenide *(InGaAs)* detector and an integral preamplifier. The interference filters used to reject light at the excitation wavelength allowed collection of Stokes

Raman scatter greater than 200 cm^{-1} shift. The laser reference frequency was set to 9396 cm^{-1} in all experiments and the mirror drive speed chosen as 0.1 cm s^{-1}.

The sample was mounted on a sample slide with the transverse surface of the wood block perpendicular to the incoming laser beam. The focus of the sample was adjusted, using the X-knob of the sample stage control, until the monitored energy was maximized. Focus adjustment was made only once for each sample. Spectra were collected using a laser intensity of 200 mW for all samples.

Infrared analyses

IR spectra were recorded using a Perkin-Elmer Fourier transform infrared 2000X spectrometer. Spectra were collected by the diffuse-reflectance method using the Perkin-Elmer DRIFT accessory. The wood samples were pulverized using a diamond paper disk, and diluted with oven-dried spectroscopic grade KBr. One hundred scans at 4 cm^{-1} resolution were collected. A reference measurement was obtained with the use of pure powdered KBr. DRIFT-spectra were plotted as the Kubelka-Munk *(KM)* function.

Chemicals

Authentic pinosylvins (L. Lundgren, SLU, Uppsala) from pine heartwood mainly containing *trans*-pinosylvin, and pure *trans*-pinosylvin (Phero Tech Inc., B. C., Canada) were used as standards. As resin acid standard a pine oil resin of known composition was used (Bergvik Kemi, Sandarne).

RESULTS AND DISCUSSION

FT-Raman and FT-IR spectroscopy are two complementary techniques suitable for compositional mapping of polymer surfaces like wood, the latter method being more frequently utilized.[10-12] The complex chemical composition of wood produced an IR spectrum which showed rather broad bands, and made it difficult to identify features of the IR spectra which could be attributed to a specific chemical constituent. The Raman lines were usually narrower and the fluorescence problem observed with a visible laser was avoided by the use of the NIR laser. The present study concentrated on the spectral interval from 1800 cm^{-1} to 900 cm^{-1} because in this region the most characteristic absorptions from the wood extracts were found.

The strongest band in a typical DRIFT-spectrum was observed around 1050 cm^{-1} indicating that the DRIFT technique, unlike Raman scattering, detected more C-O stretching and CO-H bending vibrations (Fig. 1). Visual comparison of the IR spectra showed no obvious difference between heartwood and sapwood. However, a substraction of the spectrum of the sapwood from that of the heartwood showed that the two spectra were not identical (Fig. 2). The bands at 1596 cm^{-1} and 1052 cm^{-1} were particularly evident, although it should be mentioned that the substraction factor was chosen to be unrealistically large. It may be noticed that the strong C=O band at 1699 cm^{-1} in the resin spectrum (Fig. 2) was absent in the difference spectrum, although resin acids are much more abundant in heartwood than in sapwood. Infrared spectra of both heartwood and sapwood showed an ester carbonyl vibration at 1739 cm^{-1} (Fig.1). In all samples investigated, the intensity of this band was slightly stronger for the sapwood part than for the heartwood. The absence of the acid carbonyl vibration at 1699 cm^{-1} in the difference spectrum and the existence of an ester C=O vibration in the pine wood samples indicated that the resin acids of the heartwood might be estrified.

In Figure 2, the spectrum of the pinosylvin standard is also included. Pinosylvin only occurs in the heartwood and should therefore be the possible cause for the observed spectral differences between the heartwood and the sapwood. Although the IR bands at 1596 cm^{-1} and 1507 cm^{-1} also appeared for

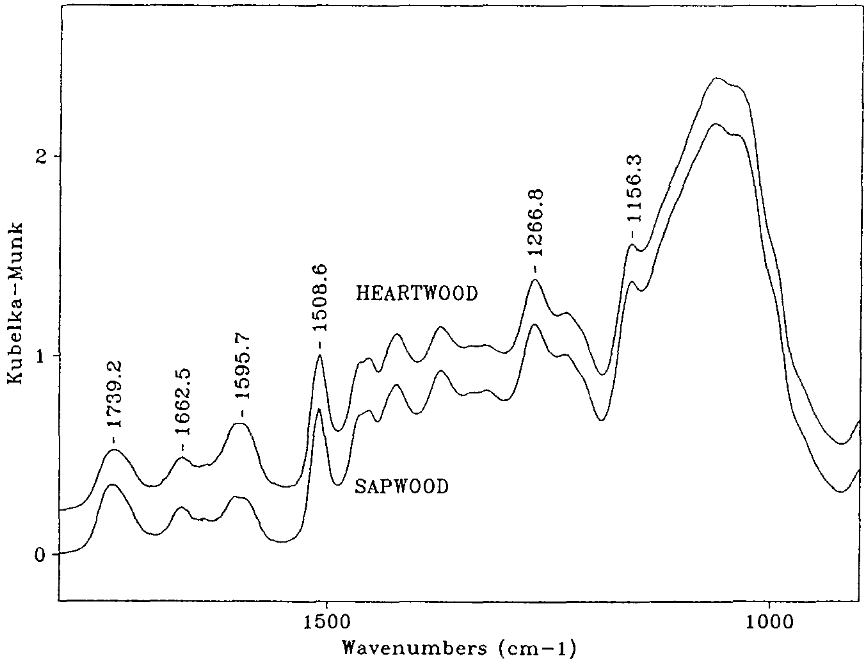

FIGURE 1. DRIFT spectra of heartwood and sapwood from Scots pine in the spectral region 1800 cm^{-1} - 900 cm^{-1}. The heartwood spectrum has been offset in order to separate the two traces.

pinosylvin, the difference spectrum reflected many other chemical differences between the heartwood and the sapwood.

Based on these results it should be possible to use IR spectra to differentiate between heartwood and sapwood by using the subtraction procedure. However, the similarities between the IR spectra suggest that a multivariate statistical approach would be useful in discriminating heartwood from sapwood.

Raman spectra of heartwood and sapwood of Scots pine are shown in Figure 3. The spectra are typical since at least ten different sample points on each side of the heartwood-sapwood boundary of ten wood samples showed similar spectra. There were at least two distinct spectral differences between heartwood and sapwood,

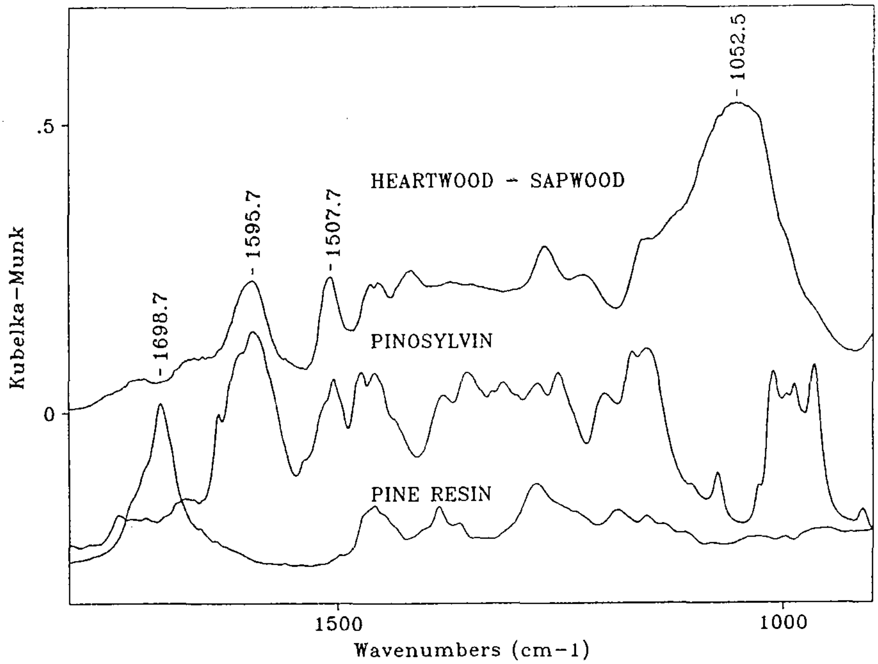

FIGURE 2. Difference spectrum of heartwood - sapwood in the spectral region 1800 cm^{-1} - 900cm^{-1}. DRIFT spectra of pinosylvin (pinosylvin + pinosylvin monomethylether) and resin extracted from Scots pine are included. The spectra have been shifted along the intensity scale for clarity.

visualized at 1636 cm^{-1} and at 999 cm^{-1}. Neither of these lines appeared in the spectrum of the sapwood. Therefore NIR-Raman spectroscopy seems to be more suitable than IR spectroscopy to discriminate between wood tissues with different concentrations of pinosylvins. The most prominent absorption in the spectrum was at 1600 cm^{-1}. The most intense lines in the Raman spectrum of pinosylvin standard were located at 1636 cm^{-1}, 1598 cm^{-1} and 997 cm^{-1} (Fig. 4). None of these lines appeared in the spectrum of the resin standard. The absorption line at

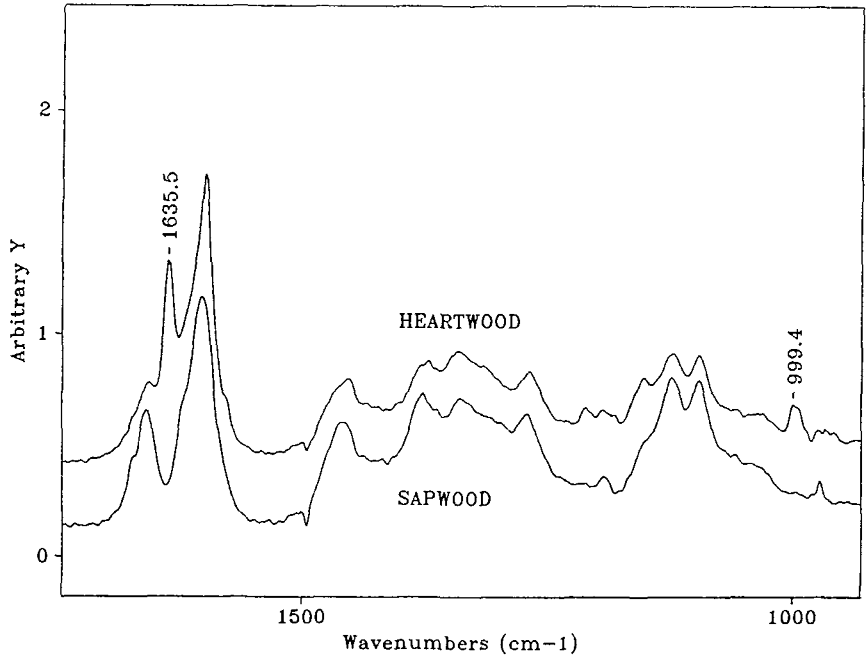

FIGURE 3. NIR FT-Raman spectra of sapwood and heartwood (offset for clarity) of Scots pine.

1598 cm^{-1} is assigned to the aromatic ring stretch, while the strong absorption at 1636 cm^{-1} is due to the olefinic C=C stretch.[13] The two pinosylvin standards used resulted in identical Raman spectra.

The difference spectrum, where the Raman spectrum of sapwood was subtracted from the spectrum of heartwood, was rather similar to the Raman spectrum of pinosylvin, especially in the spectral region 1650 cm^{-1} - 1550 cm^{-1} (Fig. 5). As a result of different chemical environments, extracted and native pinosylvin may not be expected to show identical Raman spectra. Therefore, the observed spectral differences are probably caused by the chemical environment of

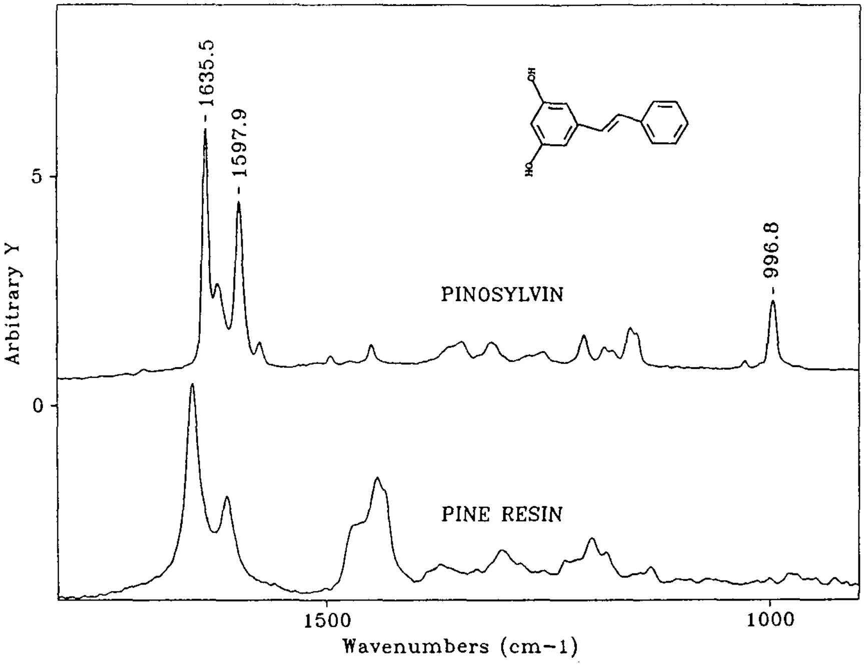

FIGURE 4. NIR FT-Raman spectra of resin extracted from Scots pine and pinosylvin (offset for clarity). The structure of pinosylvin is included.

pinosylvin in the heartwood. A further support for this suggestion was obtained by adding an acetone solution of pure pinosylvin to a piece of wood. After solvent evaporation, both sapwood and heartwood showed an increased absorption at 1636 cm^{-1} (Fig. 6). The original spectra from the heartwood coincide and were increased by the absorption due to added pinosylvin (not shown in Fig. 6). This strongly supports our suggestion that the Raman line obtained for heartwood at 1636 cm^{-1} originates from pinosylvin.

The correlation between Raman spectra of heartwood and the amount of pinosylvin measured by GC will be the subject of a future report.

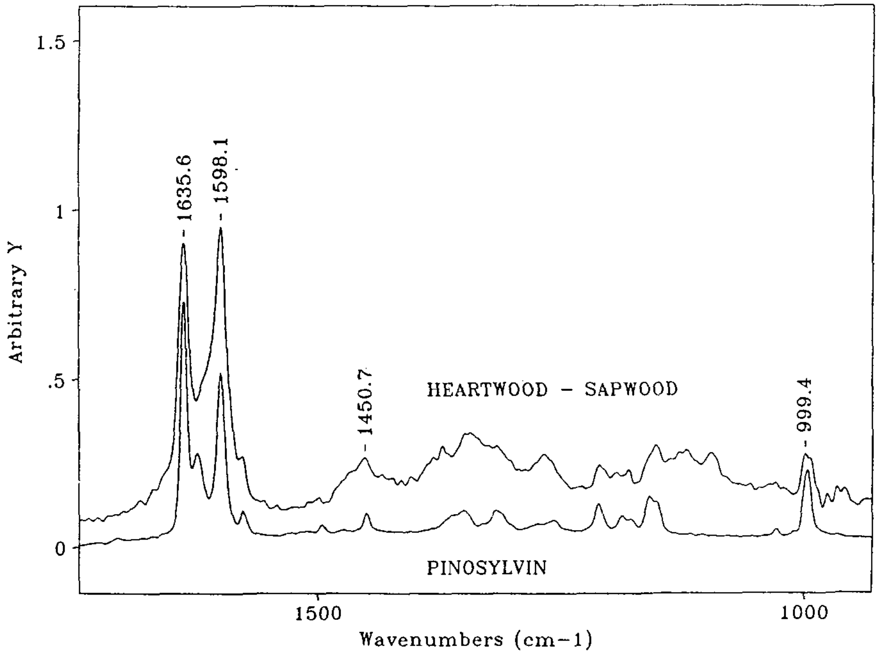

FIGURE 5. The difference between the two Raman spectra in Fig. 3. (heartwood - sapwood; offset for clarity), compared with the spectrum of pinosylvin extracted from Scots pine.

CONCLUSIONS

NIR FT-Raman spectroscopy seemed to be a suitable method to detect pinosylvins in the solid wood of Scots pine. The spectral differences between sapwood and heartwood, as reflected by Raman spectra, were mainly caused by pinosylvins.

The similarity between FT-IR spectra from heartwood and sapwood suggests a statistical approach which identifies spectral charateristics that are correlated with heartwood and sapwood, respectively.

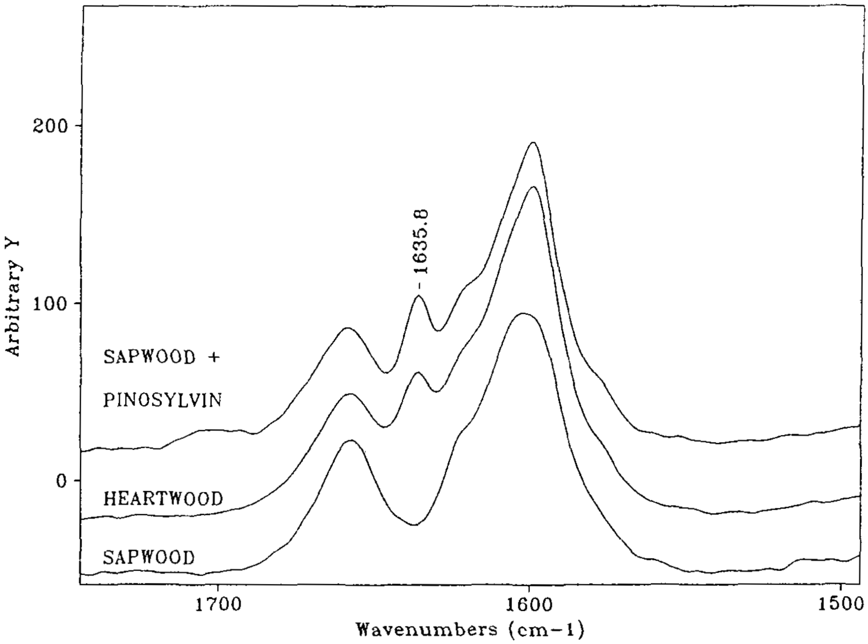

FIGURE 6. Raman spectra of sapwood with and without addition of pinosylvin standard. The Raman spectrum of heartwood is included for comparison.

Since pinosylvins are important compounds, causing differences in durability between the heartwood and sapwood of Scots pine, fast and reliable methods for classifying wood products according to their pinosylvin content would, therefore, in a future, be of significant practical value for users of wood . The sensitivity of NIR-Raman spectroscopy also makes it a unique tool in wood anatomical and biochemical research, e.g. for the detection of pinosylvins in small wood samples like the boundary region between heartwood and sapwood. Such investigations are already in progress in our laboratories and the results will be the subject of a future report.

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