

The similarity of the mineral composition for *eko* and Kaopectate suggests that similar purposes are achieved by their consumption. The extent to which the many different ethnic groups in West Africa are aware of the antidiarrheal properties of *eko* is uncertain. The fact that so many medicinal preparations in the village of Uzalla use *eko*, however, supports the notion that the therapeutic qualities of the clay are recognized by those who supply it to the West African market system and possibly by those who purchase it.

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## Raman Microprobe Evidence for Lignin Orientation in the Cell Walls of Native Woody Tissue

**Abstract.** Raman microprobe spectra from the secondary wall of earlywood tissue from *Picea mariana* (black spruce) reveal evidence of the orientation of lignin relative to the plane of the cell wall. In most instances, the aromatic rings of the phenyl propane structural units are parallel to the plane of the cell-wall surface.

Among the structural components of plant cell walls, only cellulose has been well characterized with respect to its molecular organization within native tissue (1, 2). In this work we report what we believe to be the first direct evidence for the orientation of components of lignin relative to the morphological features of native woody tissue.

A central problem in studies of plant cell walls is that the methodologies for investigating morphology, namely, electron and optical microscopy, are very limited in their capacity to develop information concerning molecular structure. On the other hand, procedures traditionally used for exploring the molecular structure of cell-wall components require prior isolation of the components by extractive procedures that are frequently destructive of morphological features (3).

The Raman microprobe offers an opportunity to bridge this gap through spectroscopic investigation of individual morphological features in unperturbed plant tissue. The microprobe permits acquisition of Raman spectra from domains as small as 1  $\mu\text{m}$  (4, 5). But its greatest asset in the present context is that molecular orientation within these domains can be established from analysis of the

polarization of Raman scattered light (6, 7).

The particular question that we addressed was the possibility that lignin molecules are oriented with respect to the geometry of the cell walls in native woody tissue. Such information would be helpful for a better understanding of its function. Although some anisotropy in the structure of lignin had been postulated on the basis of measurements of ultraviolet dichroism (8), these measurements were later attributed to form dichroism rather than to molecular orientation (1). The possibility of lignin orientation is suggested by the high degree of organization of cellulose as well as by evidence for orientation of the hemicelluloses in cell walls (9, 10). Our experiments were specifically designed to establish whether the aromatic rings of the phenyl propane units in lignin molecules had preferential orientations with respect to the plane of the cell-wall surface. In the work reported here attention was focused on the secondary wall in earlywood tissue from *Picea mariana* (black spruce).

The microprobe system consisted of a Jobin Yvon Ramanor HG2S equipped by the manufacturer with a Nachet microscope modified for coupling to the spec-

trometer and designed to provide imaging on a glass screen. The microscope serves the dual functions of focusing the exciting laser beam on the domain of interest and of gathering the Raman scattered light and imaging it at the entrance slit of the monochromator. The objective used in the present study was a  $\times 100$  (numerical aperture 1.18) liquid-immersion objective corrected for water. The exciting radiation was the 5145-Å line of an argon ion laser. The system and methodology are described in detail elsewhere (11). Our work and that of others (12) indicate that the spectra arise from domains approximately 1  $\mu\text{m}$  in diameter and 0.5  $\mu\text{m}$  deep.

The woody tissue was sectioned both longitudinally and transversely. The sections, approximately 30  $\mu\text{m}$  thick, were mounted on slides approximately 2 by 3 cm, held in place by cover slips through the centers of which small holes had been drilled. Each slide was then placed in a small flat-bottomed beaker, and  $\text{D}_2\text{O}$  was added to a depth of approximately 1 cm. After the liquid-immersion objective was brought close to the sample, the  $\text{D}_2\text{O}$  was covered with a thin layer of mineral oil to limit both evaporation and exchange with atmospheric moisture. Immersion in  $\text{D}_2\text{O}$  limited laser-induced fluorescence (11), and the  $\text{D}_2\text{O}$  bands provided a convenient internal reference for intensity measurements. The possibility of spectral effects arising from extractives, which constitute less than 1 percent of the secondary wall, was excluded on the basis of comparisons of spectral features from extracted and unextracted tissue.

The central point of the experiments was to compare spectra recorded with the polarization of the incident laser beam parallel and perpendicular to the plane of the cell wall. In order to avoid complications associated with the dichroism inherent in the optics of the microscope and the monochromator, a polarization scrambler was inserted in the path of the Raman-scattered light at the point of coupling of the microscope to the monochromator, and we obtained the different spectra by rotating the sample about the optical axis of the microscope. This was possible because the microscope was equipped with a rotating stage that had been precisely aligned.

We carried out the alignment of the stage by observing a micrometer slide and adjusting the stage so that it could be rotated without translation. At the higher magnifications, small amounts of residual translations could occur but these were readily compensated for by micro-

translation adjustments. The samples were carefully observed during the rotations and remained in sharp focus throughout. Thus any vertical translation is ruled out. The likelihood of vertical translation accompanying the rotation was also excluded by the invariance of the D<sub>2</sub>O bands with rotation when they were measured above a micrometer slide, over a period short enough that the drift in laser power output was insignificant.

The intensity difference between the D<sub>2</sub>O peaks in spectra recorded in the parallel and perpendicular modes, as in Fig. 1, A and B, reflect the drift of laser power output over the long periods of data acquisition necessary to attain spectra with reasonable signal-to-noise ratios. To avoid distortion of the relative intensities of the spectral features, the spectra were recorded by summation of many spectral scans short enough (40 minutes) that drift in laser power was insignificant within each individual scan.

Figures 1 and 2 show representative spectra obtained from a longitudinal and a transverse section, respectively. The key observations are the relative reductions in the intensity of the 1600-cm<sup>-1</sup> spectral band recorded with polarization of the exciting radiation perpendicular to the cell wall. The 1600-cm<sup>-1</sup> band has been shown to be due to a symmetric stretching of the C-C bonds of the aro-

matic ring wherein two bonds across the ring from each other vibrate in opposite phase to the other four C-C bonds (13). It has also been demonstrated that in aromatic rings with substitution patterns typical of those in lignin the 1600-cm<sup>-1</sup> band tends to be the most intense in the spectrum (14).

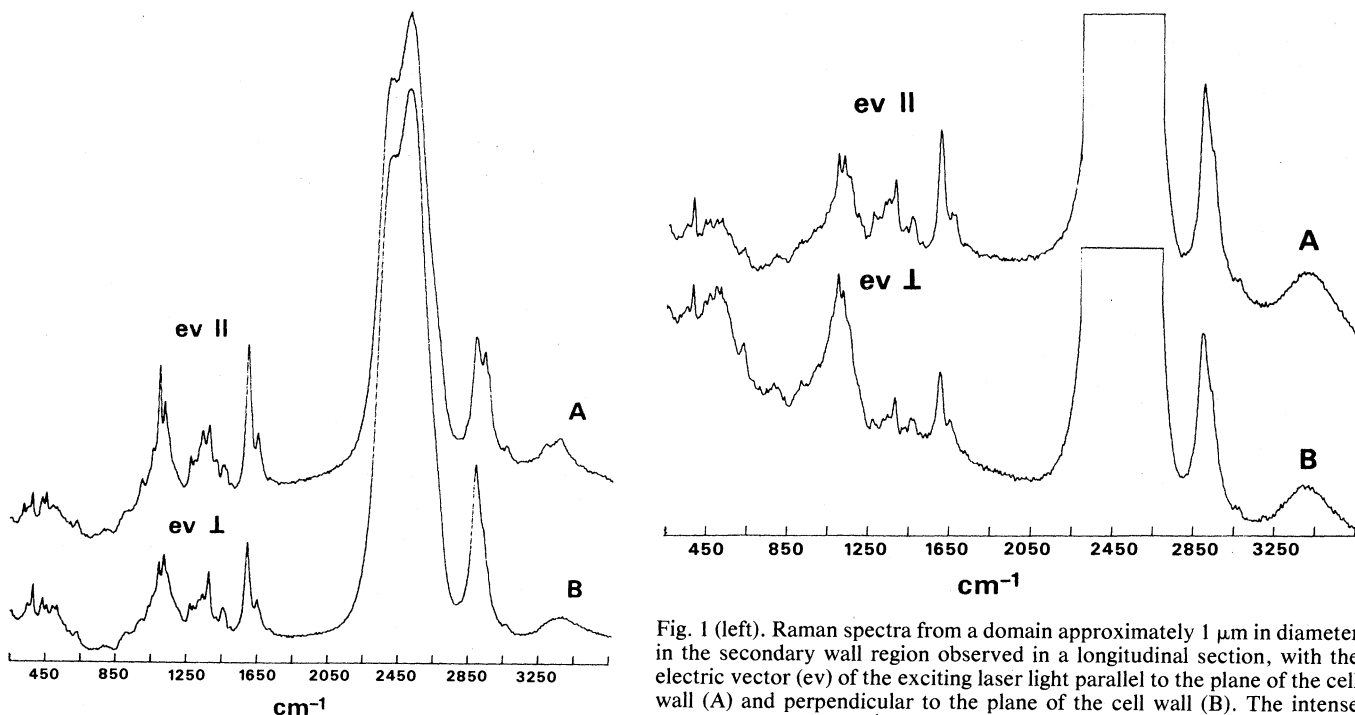
The decline in relative intensities of the 1600-cm<sup>-1</sup> bands in the spectra with polarization perpendicular to the cell wall indicates that the aromatic rings are preferentially oriented in the plane of the cell wall. This interpretation is supported by the observation that the 1650-cm<sup>-1</sup> band follows a pattern similar to that of the 1600-cm<sup>-1</sup> band. The 1650-cm<sup>-1</sup> band is due to  $\alpha$  carbonyl groups that are coplanar with the adjacent aromatic ring.

The high degree of orientation of cellulose in the cell walls (1, 2, 15) is also reflected in the spectra of Figs. 1 and 2. The 2900-cm<sup>-1</sup> band due to the methine C-H stretch changes both in shape and in intensity when the polarization of the exciting radiation relative to the plane of the cell wall is altered. The changes in the 1098-cm<sup>-1</sup> skeletal band of cellulose are also consistent with the high degree of alignment of the cellulose. Finally, the differences in the OH stretching region between 3100 and 3600 cm<sup>-1</sup>, particularly in Fig. 1, very likely reflect alignment of crystalline cellulosic domains with the chain axes parallel to the cell axis. The

spectrum in Fig. 1A, recorded with the incident beam perpendicular to the chain direction but with the electric vector parallel to the chain direction, includes two peaks superimposed upon the broader band in this region. These peaks are probably due to intramolecularly hydrogen-bonded OH groups that are parallel to the chain. Such peaks are not seen in the other three spectra reported here because, for all three, the electric vector of the exciting radiation is perpendicular to the chain direction. The OH bands associated with other constituents of native woody tissue are usually quite broad because of intermolecular hydrogen bonding.

Although the spectra shown in Figs. 1 and 2 are representative of the spectra most often observed, the spectra recorded in some other locations showed a lower degree of preferential orientation. In one or two exceptional cases the spectra showed more intense 1600-cm<sup>-1</sup> bands in the perpendicular polarization mode. This suggests the occurrence of nodes in the orientation of lignin not unlike those known to occur for cellulose in some cell walls (16). The suggestion of nodes, however, is speculative at this time, and further investigation is clearly in order.

We have carried out similar although more limited observations on tissue from *Pinus taeda* L. (loblolly pine). The re-



spectra from a domain approximately 1  $\mu$ m in diameter in the secondary wall region observed in a cross section, with the electric vector of the exciting laser light parallel to the plane of the cell wall (A) and perpendicular to the plane of the cell wall (B). The D<sub>2</sub>O bands are truncated in this plotting of the spectra.

sults fit the pattern outlined for tissue from *Picea mariana*, but it is not possible on this basis to draw conclusions concerning the degree to which our findings are representative of native woody tissue in general.

The most significant of our findings, we believe, is not so much that the orientation of the phenyl ring is most often preferentially in the plane of the cell wall but that the lignin is more highly organized at the molecular level than had heretofore been recognized.

A mapping of variations in molecular orientation throughout an individual cell would make possible detection of nodes in molecular organization and their relation to cell morphology. Such a mapping is not feasible at present, however, because the time needed to acquire spectra such as those in Figs. 1 and 2 is approximately 8 hours. It is anticipated that a new instrumental design incorporating multichannel detection will make it possible to acquire similar spectra in much shorter intervals. More comprehensive studies of the pattern of organization of lignin will then be possible.

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## Early Biochemical Effects of an Organic Mercury Fungicide on Infants: "Dose Makes the Poison"

**Abstract.** *Phenylmercury absorbed through the skin from contaminated diapers affected urinary excretion in infants in Buenos Aires. The effects were reversible and quantitatively related to the concentration of urinary mercury. Excretion of  $\gamma$ -glutamyl transpeptidase, an enzyme in the brush borders of renal tubular cells, increased in a dose-dependent manner when mercury excretion exceeded a "threshold" value. Urine volume also increased but at a higher threshold with respect to mercury. The results support the threshold concept of the systemic toxicity of metals.  $\gamma$ -Glutamyl transpeptidase is a useful and sensitive marker for preclinical effects of toxic metals.*

Phenylmercury compounds belong to a broad class of aryl and alkoxyl aryl mercurials that have worldwide use as fungicides, contraceptive spermicides, and disinfectants. Despite the fact that human exposure has been extensive, quantitative information on dose-effect relations has not, to our knowledge, been reported for this important class of organic mercury compounds.

A massive exposure of infants to phenylmercury in Buenos Aires, discovered in spring 1980 (1), provided an opportunity to obtain quantitative information on the human toxicology of this class of mercurials. Three infants were diagnosed as having the childhood disease acrodynia, or pink disease. In the past this disease was associated with the use of mercury in teething powders (2). This practice ceased around 1950, and only one case of acrodynia was reported between 1950 and 1963 (3). In 1980 the clustering of a few cases of acrodynia at the same time and place alerted pediatri-

cians to the possibility of mercury contamination. Analysis of infant urine samples revealed alarmingly high mercury concentrations. The source was discovered to be commercial diaper services, which had treated the cloth diapers with phenylmercury fungicide. About 7,000 to 10,000 babies had been exposed in this way. The prevalence of acrodynia, even in mercury-exposed babies, is very low—on the order of one per thousand (4)—and this proved to be the case in Argentina (5).

We studied 509 exposed infants (6) and 166 matched controls. The infants received a clinical examination and urine samples were obtained for clinical chemistry tests, including mercury analysis. The urine samples were collected in containers that were free of mercury and that did not absorb mercury from urine. Portions of each sample were analyzed for total mercury (7), creatinine (8),  $\gamma$ -glutamyl transferase ( $\gamma$ -GT) activity (9), and total protein (10).

The form of mercury to which the infants were exposed was confirmed by chemical analysis of diapers treated by the commercial services (11). Samples of head hair from the infants also contained phenylmercury. A number of urine samples were analyzed for total and inorganic mercury by the Magos procedure (12). Inorganic mercury accounted for over 90 percent of the total mercury, as expected from previous studies of animals exposed to phenylmercury (13).

The first urine sample was collected usually within days and always within 1 month after the end of exposure. Figure 1A depicts the distribution of total mercury concentrations in the first urine samples from exposed and control infants. The median for the exposed group is approximately 20 times greater than that for the control group. The control values are comparable with reported values in the unexposed adult population (14).

Tests for proteinuria were all negative. It was decided, therefore, to measure  $\gamma$ -GT activity, since previous reports have

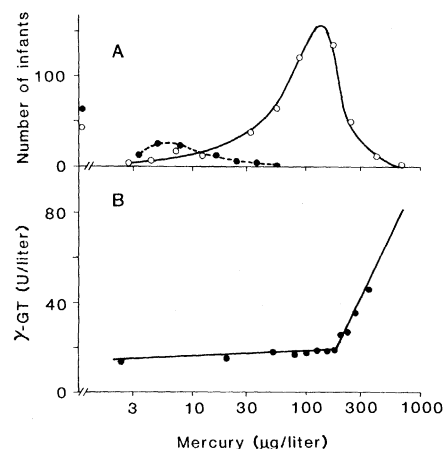


Fig. 1. (A) Distribution of mercury concentrations in the first samples of urine from exposed (○) and control (●) infants. (B) Relation between  $\gamma$ -GT and mercury concentrations in urine samples from infants exposed to phenylmercury. The lines were fitted by segmented linear regression analysis with nonlinear least-squares (19). Data points represent mean values for groups of infants (15 to 64 per group) and are present only to illustrate the goodness of fit.