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HISTOCHEMICAL STUDY ON HETEROGENEITY OF LIGNIN IN *EUCALYPTUS* SPECIES II. THE DISTRIBUTION OF LIGNINS AND POLYPHENOLS IN THE WALLS OF VARIOUS CELL TYPES

Yoko Watanabe[1,2], Yasuo Kojima[1], Toshihiro Ona[3,4], Takayuki Asada[3], Yuzou Sano[1], Kazumi Fukazawa[1] & Ryo Funada[1,5]

SUMMARY

We examined the effects of polyphenols on the analysis of lignin by histochemical methods, namely, the Mäule color reaction coupled with microspectrophotometry and ultraviolet microspectrophotometry, in wood of *Eucalyptus camaldulensis* and *E. globulus*. Thin sections and wood meals were extracted with solutions of alkali at different concentrations. The amounts of alkali-soluble extractives increased with increasing concentrations of NaOH. By contrast, there was no clear correlation between amounts of Klason lignin and the concentration of NaOH. The visible-light absorption spectra of cell walls of all woody tissues from both species changed after alkali extraction. In particular, the spectra of cell walls of vessel elements changed considerably, even when only a dilute solution of alkali was used. Ultraviolet absorption spectra did not show clear changes after extraction with alkali. These results indicate that polyphenols in cell walls affect the results of histochemical analysis. Therefore, a preliminary extraction with alkali, namely, extraction with a 1% solution of NaOH, is needed to assess the precise distribution of lignins in the cell walls of *Eucalyptus* wood by histochemical methods. The cell walls of wood fibers of *Eucalyptus camaldulensis* contained both guaiacyl and syringyl units and those of vessel walls contained mostly guaiacyl units. However, the cell walls of wood fibers in *Eucalyptus globulus* contained mainly syringyl units, while those of vessel elements contained both guaiacyl and syringyl units. Syringyl-type polyphenols, which have spectra similar to those of syringyl-type lignins, were found in the cell walls of wood fibers and vessel elements and in cell corners among wood fibers in both species of *Eucalyptus*.

Key words: *Eucalyptus camaldulensis*, *Eucalyptus globulus*, histochemistry, lignin, polyphenol, UV microspectrophotometry, cell walls.

1) Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan.
2) Hokkaido University Forests, FSC, Sapporo 060-0809, Japan.
3) Forestry Research Institute, Oji Paper, Kameyama 519-0212, Japan.
4) Graduate School of Agriculture, Kyushu University, Fukuoka 812-8581, Japan.
5) Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu-Tokyo 183-8509, Japan; corresponding author [E-mail: funada@cc.tuat.ac.jp].

INTRODUCTION

In hardwoods, lignins are composed mainly of guaiacylpropane (G) units and syringylpropane (S) units. The lignin content and S/G ratio of cell walls in hardwoods differ among cell types and among cell-wall layers in individual cells (Fergus & Goring 1970a, b; Musha & Goring 1975; Fujii et al. 1987; Lewis & Yamamoto 1990; Mellerowicz et al. 2001). Differences in S/G ratios among hardwoods affect the chemical properties of the wood and the degradation rate of lignin that is required for chemical pulping (Ona et al. 1995a). Therefore it is important to characterize the localization of guaiacyl and syringyl units in the cell walls of hardwoods in detail.

Histochemical methods, such as ultraviolet microspectrophotometry and the Mäule color reaction coupled with microspectrophotometry, allow visualization of the distribution of lignin in tissues without the destruction of cell walls (Fukazawa 1992). Ultraviolet microscopy and microspectrophotometry are useful for detection of the distribution of lignins, exploiting differences between the wavelengths of maximum absorption by guaiacyl and syringyl units. Ultraviolet photographs recorded at 280 nm and ultraviolet absorption spectra derived from thin sections reveal the localization of guaiacyl units in the cell walls of hardwoods. However, these methods are not as useful for detection of syringyl units in the cell walls because syringyl units absorb ultraviolet light much more weakly than guaiacyl units (Fergus & Goring 1970b). The Mäule color reaction provides an effective method for the detection of syringyl units as a result of differences among the colors of the *o*-quinones that are formed during the reaction (Meshitsuka & Nakano 1978). When thin sections are treated by the Mäule color reaction, cell walls that contain syringyl units turn predominantly reddish purple, whereas cell walls that contain guaiacyl units remain yellowish in color (Watanabe et al. 1997). Yoshinaga et al. (1989) reported that the Mäule color reaction coupled with microscopic spectrophotometry allowed investigations of the localization of syringyl units and guaiacyl units in the cell walls of various different cell types in hardwoods. However, the Mäule color reaction does not allow detection of guaiacyl units in cell walls that include both types of units because syringyl units are stained deep reddish purple and yellowish guaiacyl units are obscured. Therefore, the Mäule color reaction coupled with microspectrophotometry should be useful for investigations of the heterogeneity of cell-wall lignins when performed in conjunction with ultraviolet microspectrophotometry (Wu et al. 1992; Takabe et al. 1992; Watanabe & Fukazawa 1993; Yoshinaga 1995).

We have previously investigated the heterogeneity of lignins in cell walls of all tissues of the woods of some *Eucalyptus* species by histochemical methods (Watanabe et al. 1997). *Eucalyptus* is important commercially and it is planted in many countries as a pulp resource because of its rapid growth and ease of adaptation to various environmental conditions. The characteristics of lignins in *Eucalyptus* woods, as determined by chemical analysis, have been studied extensively. Bland et al. (1950) reported that the syringaldehyde/vanillin ratios of twelve species, as determined by nitrobenzene oxidation, differed considerably among species. Kawamura and Bland (1967) noted that the ratios of syringaldehyde to vanillin of five *Eucalyptus* species that had been collected from regions with different climates were related to the place of origin of the

sample trees. Ona et al. (1997) reported large within-tree variations in lignin content and lignin monomeric composition, such as the S/G ratio, in *Eucalyptus camaldulensis* and *E. globulus*. These results suggest differences in the distribution of lignins in cell walls among *Eucalyptus* species. However, to our knowledge no details of the histochemical analysis of lignins in *Eucalyptus* have been reported.

Eucalyptus woods generally contain high contents of polyphenols (Hillis 1962). Bland and Hillis (1969) pointed out that the absorption of ultraviolet light by polyphenols interfered with the visualization of lignins in cell walls in thin sections of some species of *Eucalyptus* by microspectrophotometry. Similar effects of polyphenols on the analysis of lignins by ultraviolet microscpectrophotometry have been observed in several species (Bland & Hillis 1969; Imagawa & Fukazawa 1978; Wu et al. 1990; Kleist & Bauch 2001). Therefore, in *Eucalyptus*, we must consider the effects of the polyphenols on lignin analysis. However, little information has been reported about the effects of alkali-soluble extractives, namely polyphenols, on the Mäule color reaction. In a preliminary study, we examined the effects of polyphenols on lignin analysis in *Eucalyptus* species by microspectroscopy and observed that alkali-soluble extractives caused inaccurate measurements of lignin distribution and of S/G ratios (Watanabe et al. 1997).

The goal of the present study was to clarify the effects of alkali-soluble extractives on the histochemical analysis of lignins, in particular by the Mäule color reaction, in thin sections and in wood meal, and to determine the precise distribution of lignins in *Eucalyptus* woods. We used *Eucalyptus camaldulensis* and *E. globulus* because both species are important as raw materials for pulp and paper (Ona et al. 1995a) and they contain different types of polyphenols (Hillis 1962, 1972).

MATERIALS AND METHODS

Samples

The samples used in this study were collected from 14-year-old trees, one each of *Eucalyptus camaldulensis* and *E. globulus* growing in western Australia. Several blocks ($3 \times 3 \times 3$ cm^3), including the outermost sapwood, were cut from stems at breast height. The colored heartwood was removed from *E. camaldulensis* samples (Ona et al. 1995b).

Thin sections and wood meal were prepared from the blocks. For recordings of visible-light absorption spectra after the Mäule color reaction and of ultraviolet absorption spectra, we cut 10 μm transverse sections and 50 μm radial sections. For measurements of Klason lignin content, about 3 g of wood meal, from 40 to 80 mesh, was prepared. Wood meal and thin sections were extracted with a mixture of ethyl alcohol and benzene (1 : 2, v/v) for 6 h. Samples that had been extracted in this way are referred to as control samples in this report.

Alkali extraction

Thin sections and wood meal that had been extracted with a mixture of ethyl alcohol and benzene were extracted with solutions of NaOH in an autoclave. Alkali extraction was performed under the following conditions: concentration of NaOH, 0.1, 0.4, 1.0,

4.0 or 10.0%; temperature, 105 °C; and duration, 60 min. Samples for alkali extraction weighed approximately 0.05 g in the case of thin sections and 0.5 g in the case of wood meals. After alkali extraction, samples were cooled and liquid was decanted through a ground-glass filter (pore size: 40–100 μm). Then samples were washed successively with distilled water, 10% acetic acid and distilled water. Contents of alkali-soluble extractives were calculated from weights of residues of wood meal that had been extracted with a mixture of alcohol and benzene (1 : 2, v/v).

Klason lignin contents

Klason lignin contents were measured according to the Klason method (Dence 1992). We added 72% sulfuric acid to wood meal that had been extracted with alkali. Each sample was stirred frequently for 2 h. We added distilled water to adjust the total volume of the solution for the sample (H_2SO_4 concentration of 3%). The solution was boiled for 4 h, maintaining constant volume by use of a reflux condenser, and then filtered through a ground-glass filter. Residues were washed with hot water and dried in an oven at 105 ± 3 °C. Lignin content was calculated from the weight of the oven-dried residue. Acid-soluble lignins were not quantified in this study.

Recordings of visible-light absorption spectra after the Mäule color reaction

Visible-light absorption spectra were recorded by the methods described by Wu et al. (1990). Transverse sections of 10 μm thickness, after alkali extraction, were immersed in an aqueous solution of 1% $KMnO_4$ for 5 min and then washed three times in distilled water. They were then immersed for 1 min in 3% HCl and washed again in distilled water. They were mounted on glass slides in drops of a saturated solution of ammonium hydroxide. Subsequently, sections were covered with coverslips and visible-light absorption spectra were recorded with a microspectrophotometer (UMSP-80; Carl Zeiss, Jena, Germany). Because the intensity of staining decreased gradually after the Mäule color reaction (Takabe et al. 1992; Yoshizawa et al. 1999), measurements were made within 20 min.

We recorded the visible-light absorption spectra from 400 to 700 nm of the middle layer (S_2) of the secondary walls of wood fibers, vessel elements, ray parenchyma cells and cell corners of wood fibers at intervals of 5 nm. The circular diaphragm for measurements was 1 μm in diameter and the band width of the illumination-side monochrometer was 5 nm. Samples from both species of *Eucalyptus* had false rings (Ona et al. 1995a). Therefore we selected five cells except in the case of latewood-like cells that had thick walls and small diameters and averaged the five visible-light absorption spectra. When the cell walls contain both guaiacyl and syringyl lignin, the cell walls turn reddish purple in color after the Mäule color reaction and the visible-light absorption spectra have a peak around 520 nm and a valley around 440 nm. By contrast, when the cell walls contain mainly guaiacyl lignin, the color of the cell walls remains yellowish brown after the Mäule color reaction and the visible-light absorption spectra have no peak around 520 nm. To compare the intensity of maximum absorption before and after alkali extraction we calculated the ratio of the absorbance at 520 nm to that at 440 nm ($A_{520\,nm}/A_{440\,nm}$).

Recordings of ultraviolet absorption spectra

Radial sections, 50 μm thick, that had been extracted with alkali were dehydrated through an ethanol series and embedded in epoxy resin. Then 1 μm transverse sections were cut from the embedded radial sections on an ultramicrotome (Ultracut J; Reichert, Vienna, Austria). The thin sections were mounted on quartz slides, immersed in glycerine and covered with a quartz coverslip. Ultraviolet absorption spectra were recorded from the middle layer (S_2) of the secondary walls of wood fibers and vessel elements from 250 nm to 300 nm at intervals of 1 nm with the microspectrophotometer (MPM-800; Carl Zeiss, Jena, Germany) (Wu et al. 1992; Yoshida et al. 2002). The circular diaphragm for measurements was 0.5 μm in diameter and the band width of the illumination-side monochrometer was 5 nm. Ultraviolet absorption spectra were recorded from five cells of each cell type and averaged. Ratios of the absorbance at 280 nm to that at 273 nm were calculated to compare ultraviolet absorption spectra before and after alkali extraction. Ultraviolet photomicrographs of sections were taken at 280 nm (Sano & Nakada 1998; Murakami et al. 1999).

RESULTS AND DISCUSSION

Alkali-soluble extractives and Klason lignin contents

Contents of alkali-soluble extractives and Klason lignin for *Eucalyptus camaldulensis* and *E. globulus* are shown in Figure 1. The amounts of Klason lignin in Figure 1 do not include amounts of acid-soluble lignin. The contents of alkali-soluble extractives after 1% NaOH extraction for *Eucalyptus camaldulensis* and *E. globulus* were close to 18% and 15%, respectively.

Amounts of Klason lignin for *Eucalyptus camaldulensis* were higher than those for *E. globulus*. There was no clear correlation between Klason lignin content and the concentration of NaOH. Amounts of Klason lignin did not differ at different concentrations of alkali, indicating that almost no Klason lignin was dissolved by alkali extraction.

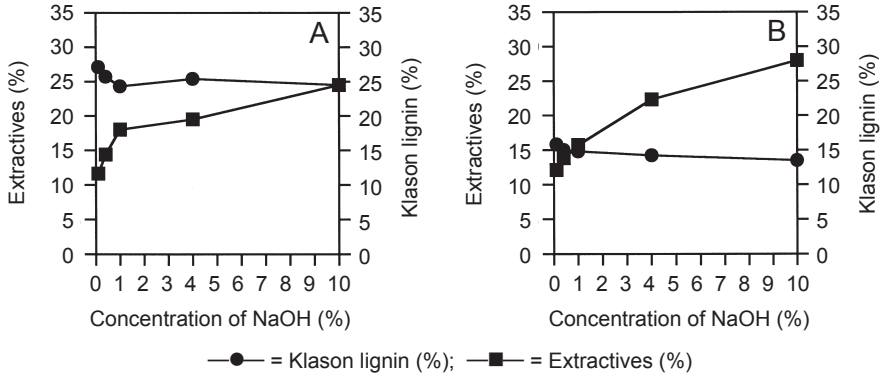

Fig. 1. Amounts of alkali-soluble extractives and Klason lignin in *Eucalyptus camaldulensis* (A) and *E. globulus* (B) after extraction with various concentrations of NaOH.

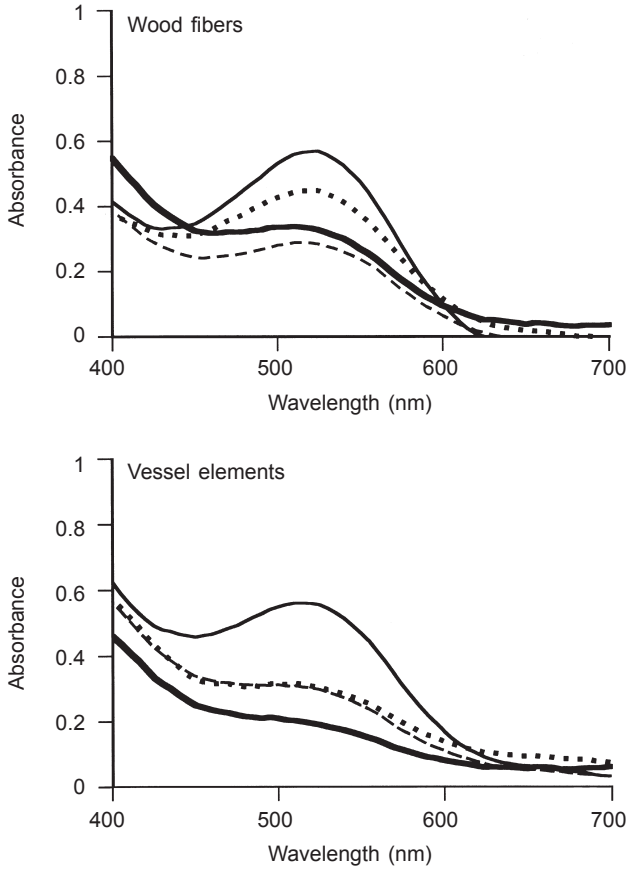

Fig. 2. Visible-light absorption spectra (mean curves), after the Mäule color reaction, of S_2 wall layers of wood fibers and vessel elements in *Eucalyptus camaldulensis* before and after alkali extraction. —— = control; ····· = extraction with 0.1% NaOH; ▬▬ = extraction with 1.0% NaOH; - - - - - = extraction with 10.0% NaOH.

However, amounts of alkali-soluble extractives tended to increase with increasing concentrations of alkali, up to 1% NaOH in both species. The amounts of alkali-soluble extractives after extraction with 10% NaOH were about twice those after extraction with 0.1% NaOH. Alkali extractives that bind to carbohydrate might be dissolved with increased concentration of NaOH.

Visible-light absorption spectra after the Mäule color reaction

Changes in visible-light absorption spectra after alkali extraction and the Mäule color reaction are shown in Figures 2 and 3. Table 1 shows the ratios of visible-light absorption spectra, after the Mäule color reaction, before and after alkali extraction. After the Mäule color reaction cell walls of both wood fibers and vessel elements of *Eucalyptus camaldulensis* became reddish purple before alkali extraction. The color of wood fiber

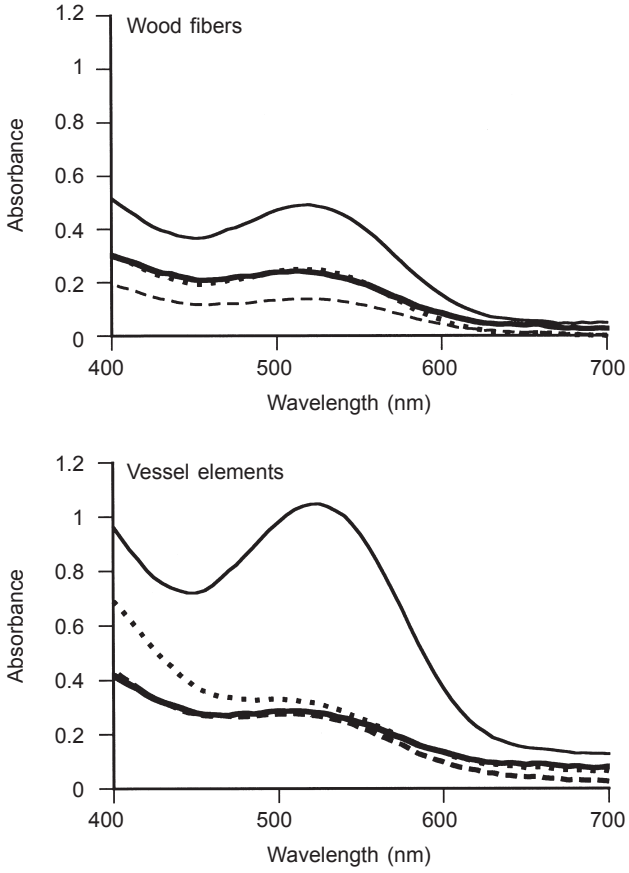

Fig. 3. Visible-light absorption spectra (mean curves), after the Mäule color reaction, of S_2 wall layers of wood fibers and vessel elements in *Eucalyptus globulus* before and after alkali extraction. —— = control; ····· = extraction with 0.1% NaOH; ▬▬ = extraction with 1.0% NaOH; - - - - - = extraction with 10.0% NaOH.

cell walls did not change after alkali extraction, but the color of vessel element cell walls changed uniformly from reddish purple to yellowish brown after alkali extraction with concentrations of more than 1% NaOH. In *Eucalyptus globulus*, the color of cell walls of both wood fibers and vessel elements after the Mäule color reaction became reddish purple before alkali extraction and did not change after alkali extraction. Figure 2 shows absorbance of visible light by cell walls of wood fibers in *Eucalyptus camaldulensis* decreased after alkali extraction and the maximum absorption also decreased. However, the ratio $A_{520\,nm}/A_{440\,nm}$ did not fall below 1.0, even after extraction with 10% NaOH (Table 1). These results indicated that the cell walls of wood fibers in *Eucalyptus camaldulensis* still contained syringyl lignin after extraction of syringyl-type polyphenols with alkali. In vessel element cell walls of *Eucalyptus camaldulensis*, absorbance decreased considerably after alkali extraction. Moreover, ratios of relevant wavelengths

Table 1. Ratios ($A_{520\,nm}/A_{440\,nm}$) calculated from visible-light absorption spectra, after the Mäule color reaction, before and after alkali extraction for *Eucalyptus camaldulensis* and *E. globulus* (n = 5). — C.C. = cell corners among wood fibers; Ray = ray parenchyma cells.
Note: The ratios are ratios of the absorbance at 520 nm to that at 440 nm ($A_{520\,nm}/A_{440\,nm}$).

Eucalyptus camaldulensis	Wood fibers	Vessel elements	C.C.	Ray
Control	1.70	1.20	1.17	0.94
0.1% NaOH	1.45	0.84	0.80	1.20
0.4% NaOH	1.34	0.95	1.03	1.04
1.0% NaOH	0.94	0.70	0.80	1.10
4.0% NaOH	1.45	0.72	0.95	1.04
10.0% NaOH	1.12	0.82	0.95	1.08
Eucalyptus globulus	Wood fibers	Vessel elements	C.C.	Ray
Control	1.31	1.43	1.29	0.83
0.1% NaOH	1.20	0.75	0.89	0.89
0.4% NaOH	1.06	0.76	0.87	1.07
1.0% NaOH	1.07	0.94	0.87	0.92
4.0% NaOH	1.25	0.88	1.00	1.22
10.0% NaOH	1.10	0.93	0.81	1.34

fell below 1.0 after extraction with 0.1% NaOH (Table 1). The visible-light absorption spectra of vessel elements had a small peak around 520 nm after alkali extraction, up to 0.1% NaOH (Fig. 2). However, no peak was found around 520 nm after extraction with 1% NaOH. These results indicated that syringyl-type polyphenols had been extracted. Therefore, the cell walls of the vessel elements in *Eucalyptus camaldulensis* did not contain syringyl lignin but did contain syringyl-type polyphenols uniformly.

In *Eucalyptus globulus*, the cell walls of wood fibers still contained syringyl lignin after alkali extraction (Fig. 3 and Table 1). In vessel elements of *E. globulus*, the absorbance of visible light fell considerably after alkali extraction (Fig. 3). The ratios of relevant wavelengths fell below 1.0 after extraction with 0.1% NaOH (Table 1). However, the visible-light absorption spectra had a small peak of maximum absorption at 520 nm. Thus, cell walls of vessel elements in *E. globulus* contained syringyl lignin even after alkali extraction.

The absorbance of visible light by cell corners among wood fibers in both species fell after alkali extraction (data not shown), and these results were similar to those for wood fibers. The ratios calculated from the spectra for both species were greater than 1.0 before alkali extraction and decreased after alkali extraction (Table 1). Syringyl-type polyphenols in cell corners among wood fibers might be extracted by alkali. The visible-light absorption spectra for cell walls of ray parenchyma in both species still had a peak of maximum absorption at 520 nm after alkali extraction, even when 10% NaOH was used (Table 1). Therefore, the cell walls of ray parenchyma cells contained syringyl lignin. Ray parenchyma cells of both *Eucalyptus camaldulensis* and *E. globulus* included much brownish materials after the Mäule color reaction, indicating that these were guaiacyl-type compounds. However, the brownish materials in ray parenchyma

cells of both species were dissolved by alkali. Therefore, the ratios calculated from the visible-light absorption spectra increased after alkali extraction. It is likely that these materials interfered with the recordings of the visible-light absorption spectra. Such alkali-soluble materials might be phenolic compounds (Higuchi 1976).

Our present results indicate that polyphenols, which were extracted by alkali, are uniformly present in lignified cell walls and have a considerable effect on the analysis of lignin by visible-light absorption spectroscopy, especially of cell walls of vessel elements. We reported previously that the materials dissolved by alkali solution, at 0.4% and 1.0% NaOH, might be guaiacyl-type polyphenols since they had a peak of maximum absorption at 280 nm (Watanabe et al. 1997). However, in the present study, the changes in the visible-light absorption spectra upon alkali extraction indicated that syringyl-type extractives were also dissolved by alkali. Guaiacyl nuclei absorb ultraviolet light more strongly than syringyl nuclei (Fergus & Goring 1970b). Therefore it is likely that syringyl-type extractives were not detected when previous measurements were made.

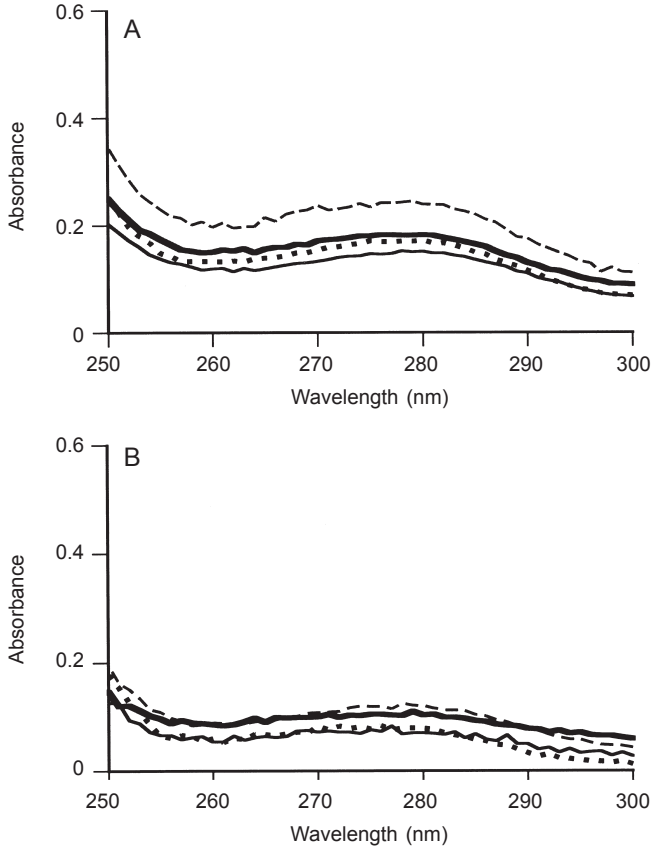

Fig. 4. Ultraviolet absorption spectra of wood fibers in *Eucalyptus camaldulensis* (A) and *E. globulus* (B) before and after alkali extraction. —— = control; ····· = extraction with 0.1% NaOH; ▬▬ = extraction with 1.0% NaOH; - - - - - = extraction with 10.0% NaOH.

Ultraviolet absorption spectra

Unlike the results of the Mäule color reaction, ultraviolet photographs did not show clear changes in the absorbance of ultraviolet light in cell walls of wood fibers and vessel elements in both *Eucalyptus* species after alkali extraction (data not shown). The cell walls of all elements were swollen after extraction with 10% NaOH. Materials in ray parenchyma cells were dissolved by alkali extraction, as in the case of the Mäule color reaction. Such materials in ray parenchyma cells absorbed ultraviolet light strongly at 280 nm.

Ultraviolet absorption spectra of cell walls of wood fibers before and after alkali extraction are shown in Figure 4. There was little difference between the ultraviolet absorption spectra of cell walls of wood fibers recorded before and after alkali extraction in both species. The ultraviolet absorption spectra of *Eucalyptus camaldulensis* ($A_{280\,nm}$ 0.18 ± 0.04) had a higher peak at 280 nm than those of *E. globulus* ($A_{280\,nm}$ 0.09 ± 0.02). In both species, the absorption of ultraviolet light by samples that had been extracted with 10% NaOH was elevated. Extractives from the brownish materials in ray parenchyma cells that have been dissolved in a solution of 10% NaOH may recombine with components of the cell wall during alkali extraction.

Table 2. Ratios of absorbance at 273 nm to that at 280 nm calculated from ultraviolet absorption spectra of wood fibers of *Eucalyptus camaldulensis* and *E. globulus* before and after alkali extraction (n = 5).

Treatment	*Eucalyptus camaldulensis*	*Eucalyptus globulus*
Control	0.94	1.03
0.1% NaOH	0.95	1.01
0.4% NaOH	—	1.06
1.0% NaOH	0.97	1.00
4.0% NaOH	1.00	1.02
10.0% NaOH	0.98	0.98

— = not determined.

Table 2 shows ratios of absorbance at 273 nm (A_{max} of syringyl units) to that at 280 nm (A_{max} of guaiacyl units) for ultraviolet absorption spectra of wood fibers of *Eucalyptus camaldulensis* and *E. globulus* and reveals the changes in absorbance of ultraviolet light after alkali extraction. In *Eucalyptus camaldulensis*, the ratios were slightly less than 1.0 and tended to increase with increasing concentrations of alkali. Although the cell walls of wood fibers of *E. camaldulensis* were stained purple by the Mäule color reaction (Watanabe et al. 1997), the ultraviolet absorption spectra indicated that these walls contained guaiacyl units. By contrast, the ratios for *Eucalyptus globulus* were slightly more than 1.0 and the changes after alkali extractions were minimal. In *E. globulus*, the ultraviolet absorption spectra indicated that the cell walls of wood fibers contained syringyl units for the most part. This conclusion reflects the results of the Mäule color reaction.

In the case of the vessel elements of both species, the ultraviolet absorption spectra revealed strong absorption at 280 nm and the ratios for cell walls of vessel elements were less than 1.0 (data not shown). These results indicated that in both species the cell walls of vessel elements contained mostly guaiacyl units. Wu et al. (1990) reported that, in five tropical hardwoods, the ratios of absorbance at 280 nm to that at 274 nm changed in all cell types, and in particular in cell walls of vessel elements and ray parenchyma cells, after alkali extraction. They concluded that alkali-soluble extractives in cell walls affected the ultraviolet absorption spectra. In *Eucalyptus* species, the lignified cell walls include considerable amounts of polyphenols (Bland & Hillis 1969; Watanabe et al. 1997). It is conceivable that alkali-soluble extractives affected the ultraviolet absorption spectra in the present study. Our results indicate that both guaiacyl- and syringyl-type polyphenols, possibly such as tannin, flavonoid and lignan, might be dissolved homogeneously in woods of the two species of *Eucalyptus*.

CONCLUSIONS

The present results showed that alkali-soluble extractives uniformly exist in cell walls in *Eucalyptus* sapwoods and influence the analysis of lignins in *Eucalyptus* woods by histochemical methods. Consequently, initial extraction with 1% NaOH might improve histochemical analysis because such extraction removes sufficient polyphenols to allow measurements of absorbance in lignified cell walls of *Eucalyptus* without interference by polyphenols.

Our histochemical analysis revealed the precise distribution of lignins and polyphenols in *Eucalyptus camaldulensis* and *E. globulus*. The cell walls of wood fibers in *E. camaldulensis* contain both guaiacyl and syringyl units and those of vessel walls consist mostly of guaiacyl units. By contrast, the cell walls of wood fibers in *E. globulus* contain mainly syringyl units and those of vessel elements contain both guaiacyl and syringyl units. In both species the cell walls of wood fibers and vessel elements, ray parenchyma cells, and the interfiber cell corners contain syringyl-type polyphenols. The brownish materials in the ray parenchyma cells of both species might be phenolic compounds that have a chemical composition similar to guaiacyl units. Removal of polyphenols by alkali prior to histochemical analysis of lignin allows more accurate characterization of lignin heterogeneity in the cell walls of *Eucalyptus* wood, which are important raw materials for the pulp and paper industries.

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REFERENCES

Bland, D. E. & W. E. Hillis. 1969. Microspectrophotometric investigation of lignin and polyphenol distribution in wood sections. Appita 23: 204–210.

Bland, D.E., G. Ho & W.E. Cohen. 1950. Aromatic aldehydes from the oxidation of some Australian woods and their chromatographic separation. Aust. J. Sci. Res., Ser. A3: 642–648.
Dence, C.W. 1992. The determination of lignin. In: S.Y. Lin & C.W. Dence (eds.), Methods in lignin chemistry: 33–61. Springer-Verlag, Berlin.
Fergus, B.J. & D.A.I. Goring. 1970a. The location of guaiacyl and syringyl lignins in birch xylem tissue. Holzforschung 24: 113–117.
Fergus, B.J. & D.A.I. Goring. 1970b. The distribution of lignin in birch wood as determined by ultraviolet microscopy. Holzforschung 24: 118–124.
Fujii, T., K. Shimizu & A. Yamaguchi. 1987. Enzymatic saccharification on ultrathin sections and ultraviolet spectra of Japanese hardwoods and softwoods. Mokuzai Gakkaishi 33: 400–407.
Fukazawa, K. 1992. Ultraviolet microscopy. In: S.Y. Lin & C.W. Dence (eds.), Methods in lignin chemistry: 110–121. Springer-Verlag, Berlin.
Higuchi, T. 1976. Biochemical aspects of lignification and heartwood formation. Wood Res. 59/60: 180–199.
Hillis, W.E. 1962. The distribution and formation of polyphenols within the tree. In: W.E. Hills (ed.), Wood extractives and their significance to the pulp and paper industry: 59–131. Academic Press, New York.
Hillis, W.E. 1972. Properties of eucalypt woods of importance to the pulp and paper industry. Appita 26: 113–121.
Imagawa, H. & K. Fukazawa. 1978. Influence of wood extractives on UV microscopy for lignin determination. Mokuzai Gakkaishi 24: 583–586.
Kawamura, I. & D.E. Bland. 1967. The lignins of Eucalyptus wood from tropical and temperate zones. Holzforschung 21: 65–74.
Kleist, G. & J. Bauch. 2001. Cellular UV microspectrophotometric investigation of Sapelli heartwood (Entandrophragma cylindricum Sprague) from natural provenances in Africa. Holzforschung 55: 117–122.
Lewis, N.G. & E. Yamamoto. 1990. Lignin: Occurrence, biogenesis and biodegradation. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41: 455–496.
Mellerowicz, E.J., M. Baucher, B. Sundberg & W. Boerjan. 2001. Unravelling cell wall formation in the woody dicot stem. Plant Mol. Biol. 47: 239–274.
Meshitsuka, G. & J. Nakano. 1978. Studies on the mechanism of lignin color reaction (12) Mäule color reaction (8). Mokuzai Gakkaishi 24: 563–568.
Murakami, Y., R. Funada, Y. Sano & J. Ohtani. 1999. The differentiation of contact cells and isolation cells in xylem ray parenchyma of Populus maximowiczii. Ann. Bot. 84: 429–435.
Musha, Y. & D.A.I. Goring. 1975. Distribution of syringyl and guaiacyl moieties in hardwoods as indicated by ultraviolet microscopy. Wood Sci. Technol. 9: 45–58.
Ona, T., T. Sonoda, K. Itoh & M. Shibata. 1995a. Studies on decision of selection indexes for quality breeding of Eucalypt pulpwood. I. Within-tree variations and the positions representing the whole-tree values of polysaccharides contents on E. camaldulensis and E. globulus. Japan Tappi 49: 724–734.
Ona, T., T. Sonoda, K. Itoh & M. Shibata. 1995b. Studies on decision of selection indexes for quality breeding of Eucalypt pulpwood. II. Within-tree variations and the positions representing the whole-tree contents of extraneous components on E. camaldulensis and E. globulus. Japan Tappi 49: 861–869.
Ona, T., T. Sonoda, K. Itoh & M. Shibata. 1997. Relationship of lignin content, lignin monomeric composition and hemicellulose composition in the same trunk sought by their within-tree variations in Eucalyptus camaldulensis and E. globulus. Holzforschung 51: 396–404.
Sano, Y. & R. Nakada. 1998. Time course of the secondary deposition of incrusting materials on bordered pit membrane in Cryptomeria japonica. IAWA J. 19: 285–299.

Takabe, K., S. Miyauchi, R. Tsunoda & K. Fukazawa. 1992. Distribution of guaiacyl and syringyl lignins in Japanese beech (Fagus crenata): Variation within an annual ring. IAWA Bull. n.s. 13: 105–112.

Watanabe, Y. & K. Fukazawa. 1993. Lignin heterogeneity of the cell walls on the genus Acer. Res. Bull. Hokkaido Univ. Forests 50: 349–389.

Watanabe, Y., K. Fukazawa, Y. Kojima, R. Funada, T. Ona & T. Asada. 1997. Histochemical study on heterogeneity of lignin in Eucalyptus species I. Effects of polyphenols. Mokuzai Gakkaishi 43: 102–107.

Wu, J., K. Fukazawa & J. Ohtani. 1990. Lignin analysis in some tropical hardwoods using ultraviolet microscopy. Res. Bull. Coll. Exp. Forests, Hokkaido Univ. 47: 353–366.

Wu, J., K. Fukazawa & J. Ohtani. 1992. Distribution of syringyl and guaiacyl lignins in hardwoods in relation to habitat and porosity form in wood. Holzforschung 46: 181–185.

Yoshida, M., H. Ohta & T. Okuyama. 2002. Tensile growth stress and lignin distribution in the cell walls of black locust (Robinia pseudoacacia). J. Wood Sci. 48: 99–105.

Yoshinaga, A. 1995. Cellular distribution of guaiacyl and syringyl lignins in oak wood analyzed by microscopic spectrophotometry. PhD thesis, Kyoto University.

Yoshinaga, A., M. Fujita & H. Saiki. 1989. Evaluation of the varieties of lignin in wood and bamboo cell walls by Mäule color reaction coupled with microscopic spectrophotometry. Bull. Kyoto Univ. Forests 61: 276–284.

Yoshizawa, N., H. Ohba, J. Uchiyama & S. Yokota. 1999. Deposition of lignin in differentiating xylem cell walls of normal and compression wood of Buxus microphylla var. insularis Nakai. Holzforschung 53: 156–160.