

RAPID COMMUNICATION

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## Proof of the presence of guaiacyl–syringyl lignin in *Selaginella tamariscina*

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Selaginellaceae are an ancient group of fern allies composed of an estimated 750 living species.<sup>1</sup> Although Selaginellaceae are primitive vascular plants, the presence of vessel elements other than tracheids in some species has been reported.<sup>2</sup> The existence of vessel elements and a characteristic lignin structure has attracted the interest of many researchers. Generally, lignin of pteridophytes is considered to be of the gymnospermous type because of the phylogenetic position of the plant. In fact, lignin of many pteridophytes consists mainly of guaiacyl nuclei in addition to a small amount of *p*-hydroxyphenyl nuclei. Interestingly, tissues of Selaginellaceae exhibit purple color (positive) in the Mäule test<sup>3</sup> and produce syringaldehyde and acetosyringone by alkaline nitrobenzene oxidation and cupric oxide oxidation,<sup>4,5</sup> respectively. The positive Mäule test of Selaginellaceae was observed in the cortex tissues rather than in the xylem in many cases.<sup>3</sup> These data suggested the presence of syringyl nuclei in the tissue of Selaginellaceae. However, detection of syringyl nuclei in the tissue of Selaginellaceae by a positive Mäule test or an alkaline nitrobenzene oxidation does not necessarily mean that

syringyl nuclei constitute lignin as a phenylpropane unit (C<sub>6</sub>-C<sub>3</sub> unit).<sup>6</sup> To date, it has not been made clear whether such syringyl nuclei that appear to be present in species of Selaginellaceae are due to lignin or structural polyphenols other than lignin.

The phenylpropane (C<sub>6</sub>-C<sub>3</sub>) units of the uncondensed type are broken into corresponding C<sub>6</sub>-C<sub>1</sub> compounds by alkaline nitrobenzene oxidation, whereas analytical acidolysis provides specific C<sub>6</sub>-C<sub>3</sub> products by the cleavage of arylglycerol- $\beta$ -aryl ether linkage.<sup>7,8</sup> If syringyl nuclei are found frequently in such C<sub>6</sub>-C<sub>3</sub> type products following acidolysis, it can afford proof of syringyl nuclei as phenylpropane units that constitute lignin, because arylglycerol- $\beta$ -aryl ether is the most abundant structure in lignin.

In this study, two pteridophytes, *Selaginella tamariscina* and *Polystichum polyblepharum*, were collected from the University Forest in Chiba (N 35° 40', W 140° 05'), of the University of Tokyo. *Selaginella tamariscina* and *P. polyblepharum* are species of Selaginellaceae and Aspidiaceae, respectively. Björkman lignin of *S. tamariscina* and *P. polyblepharum* (stem) were prepared according to Björkman's procedure after successive extraction with boiling aqueous 80% ethanol (1 h, three times) and water overnight at 40°C with shaking. The yields of Björkman lignin isolated from *S. tamariscina* and *P. polyblepharum* (stem) were 6.4% and 3.5% (of finely ground sample), respectively. The Björkman lignin was characterized by proton nuclear magnetic resonance (<sup>1</sup>H-NMR) spectroscopy and Fourier transform infrared (FTIR) spectroscopy. In addition, ground samples of these two pteridophytes and their Björkman lignins were subjected to acidolysis, alkaline nitrobenzene oxidation, and ozonation<sup>9</sup> analyses.

The total yields of alkaline nitrobenzene oxidation products of *S. tamariscina* from ground sample and Björkman lignin were 0.16 and 0.24 mol/200 g lignin, and the molar ratio of syringaldehyde to vanillin (S/V ratio) was 2.21 and 2.31, respectively (Table 1). No syringaldehyde was detected in *P. polyblepharum*. In the <sup>1</sup>H-NMR spectrum of *S. tamariscina*, a strong signal was observed at 6.6 ppm, which was absent in the spectrum of *P. polyblepharum* (Fig. 1).

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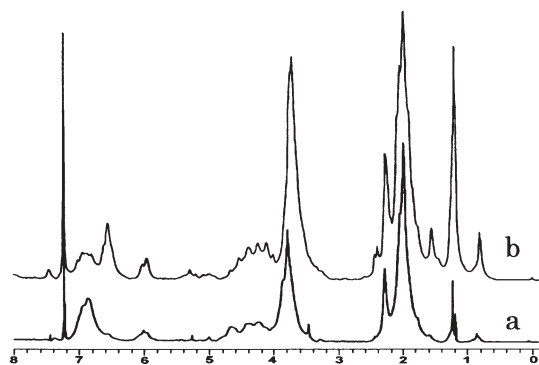
**Table 1.** Results of alkaline nitrobenzene oxidation, ozonation, and acidolysis

		<i>Selaginella tamariscina</i>		<i>Polystichum polyblepharum</i>		<i>Fagus crenata</i> Finely ground sample
		Björkman lignin	Finely ground sample	Björkman lignin	Finely ground sample	
Alkaline nitrobenzene oxidation	Total yield <sup>a</sup>	0.24	0.16	0.24	0.18	0.55
	S/V	2.31	2.21	0	0	2.70
Ozonation	E/T	1.70	2.16	0.86	1.08	2.51
Acidolysis	S/G	1.28	1.30	0	0	1.11
$S_{\text{acidolysis}}/S_{\text{nitrobenzene}}^b$		0.14	0.22	0	0	0.17

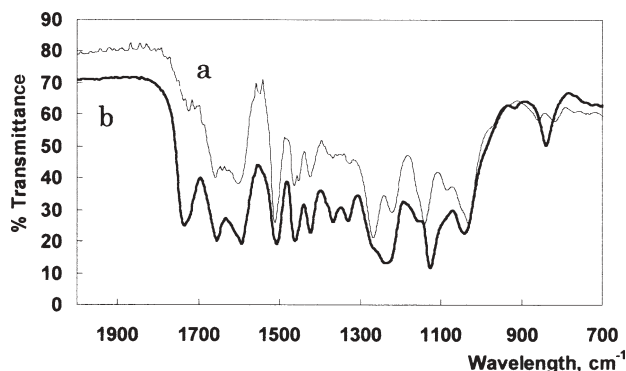
S/V, Syringaldehyde to vanillin molar ratio; E/T, *erythro/threo* ratio; S/G, syringyl/guaiacyl ratio

<sup>a</sup>Total yield measured in units of mol/200 g lignin

<sup>b</sup>Ratio of syringyl-type acidolysis products to syringaldehyde by an alkaline nitrobenzene oxidation



**Fig. 1.** Proton nuclear magnetic resonance spectra of acetylated Björkman lignin from *a* *Polystichum polyblepharum* and *b* *Selaginella tamariscina*



**Fig. 2.** Fourier transform infrared spectra of Björkman lignin from *a* *P. polyblepharum* and *b* *S. tamariscina*

The signal at 6.6 ppm is assigned to the aromatic protons of syringyl nuclei. An intensive single peak at 835 cm<sup>-1</sup> in the FTIR spectrum (Fig. 2) also suggested the presence of syringyl nuclei in *S. tamariscina*.<sup>10</sup> Furthermore, intensive absorptions in *S. tamariscina* appeared at 1130 cm<sup>-1</sup> and 1230 cm<sup>-1</sup> in the FTIR spectrum, which are also characteristic of syringyl nuclei.<sup>10</sup> On the contrary, the intensive bands in *P. polyblepharum* lignin were shown at 1030 cm<sup>-1</sup> and 1275 cm<sup>-1</sup>, which were assigned as guaiacyl nuclei (Fig. 2).

The results from alkaline nitrobenzene oxidation and spectrometric analyses not only agreed well with earlier findings<sup>3-5</sup> but also clearly demonstrated that syringyl nuclei were present as the major aromatic composition in *S. tamariscina* lignin.

The presence of arylglycerol- $\beta$ -aryl ether intermonomer linkage is essential to distinguish lignin from other polyphenols. Acidolysis of angiosperm lignin gives rise to both 4-hydroxyl-3-methoxyphenylpropanones (guaiacyl type, G) and 4-hydroxy-3,5-dimethoxyphenylpropanones (syringyl type, S), whereas that of gymnosperm lignin produces only 4-hydroxyl-3-methoxyphenylpropanones.<sup>7,8</sup> Two kinds of syringyl-type arylpropanones ( $M^+$  370) were identified by gas chromatography-mass spectrometric (GC-MS) analysis of acidolysis products obtained from Björkman lignin and ground sample of *S. tamariscina* together with two kinds of guaiacyl type arylpropanones ( $M^+$  340). These four signals were identical to those observed in *Fagus crenata*, which is a typical angiospermous species. Syringyl-type arylpropanones were absent in *P. polyblepharum*. The ratios of syringyl to guaiacyl types (S/G ratio) of acidolysis products calculated from the peak areas on the GC chromatograms were 1.30 and 1.28 for the ground sample of *S. tamariscina* and its Björkman lignin, respectively (Table 1). These values were higher than that of *F. crenata*. In addition, *S. tamariscina* exhibited a higher product ratio of syringyl-type acidolysis products to the corresponding alkaline nitrobenzene oxidation products ( $S_{\text{acidolysis}}/S_{\text{nitrobenzene}}$  ratio) than that of *F. crenata* (Table 1). Recently, Akiyama et al.,<sup>11</sup> found that lignin possesses a high *erythro/threo* ratio (E/T ratio) of arylglycerol- $\beta$ -aryl ether structures when the S/V ratio is high based on the analysis of lignin for 21 wood species. Therefore, a high E/T ratio obtained for *S. tamariscina* (Table 1) also suggests that syringyl nuclei participate in the formation of arylglycerol- $\beta$ -aryl ether structures. These results confirm that syringyl nuclei are surely incorporated into lignin as the main C<sub>6</sub>-C<sub>3</sub> type structural elements.

All results obtained in the present study indicate the presence of guaiacyl-syringyl lignin in *S. tamariscina*.

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