論文の内容の要旨

Variation of lignin β-O-4 structures in reaction woods and in *Acacia* and *Eucalyptus* species
（アテ材中およびアカシア、ユーカリ樹種のリグニンβ-O-4構造の変動）

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Introduction

The structure of lignins varies widely among different wood species.[1-3] However, the structural differences and variations are not random, but can be organized based on the type of aromatic nuclei, that is the proportion of syringyl unit (syringyl ratio or S/G ratio). It has been reported that a clear correlation between syringyl ratio and *erythro* ratio of *β*-O-4 structure was found among lignins of various wood species.[2] Some factors other than the S/G ratio, however, also possibly influence on the formation process of lignin during its biosynthesis, and would causes structural differences in lignins, which must influence on the chemical reactivity in the paper making process. The present study aimed at finding more clear correlations and exploring new structural features behind lignin structures, which would possibly be attained by narrowing down the wood-sampling variation to the same genus or the same species from different habitats, and different parts within a stem of reaction wood. In addition, unique wood species, *G. gnemon*, which interestingly contains syringyl-guaiacyl lignin even though this is a gymnosperm species, was examined for the lignin analyses to investigate whether its reaction wood is gymnosperm- or angiosperm-like. The characteristic of *G. gnemon* was evaluated based on a classification methodology developed in this study through the lignin analyses of various reaction woods focusing on the *β*-O-4 structures, which are the most predominant type of lignin linkage. Through this thesis, lignin samples were characterized by their *β*-O-4 structures, and their stereochemical structures were discussed in the context of their formation mechanisms and a factor influencing on the ratio of *erythro* and *threo* forms during lignin biosynthesis.

Experimental

Characteristics of lignin in Acacia and Eucalyptus woods

Lignin content, the proportion of the aromatic ring types (syringyl ratio), and the proportion of the diastereomeric forms for the *β*-O-4 structure (*erythro* ratio) were investigated for 28 wood samples belonging to the *Acacia* and *Eucalyptus* genera (Table 1) by Klason methods, nitrobenzene oxidation, and ozonation method, respectively.

Each wood sample in chip form (200 g) was also subjected to Kraft cooking in a batch digester using 1000 ml of cooking liquor with 28% sulfidity. Four different levels of active alkali charges were used for each wood sample in order to estimate the active alkali charge required to obtain a pulp with lignin content of Kappa 19 (approximately 2.9%) under the following cooking conditions: time taken to reach the cooking temperature: 100 min; cooking temperature: 155°C; and cooking time: 120 min.

Table 1. List of wood samples examined in this study

Sample No.	Species of genus *Acacia*	Sample No.	Species of genus *Eucalyptus*
1	*Acacia auriculiformis*	16	*Eucalyptus camaldulensis* A
2	*Acacia hybrid* ^{*1}	17	*Eucalyptus camaldulensis* B
3	*Acacia hybrid* ^{*1}	18	*Eucalyptus deglupta*
4	*Acacia hybrid* ^{*1}	19	*Eucalyptus dunii*
5	*Acacia hybrid* ^{*1}	20	*Eucalyptus globulus* A
6	*Acacia hybrid* ^{*1}	21	*Eucalyptus globulus* B
7	*Acacia hybrid* ^{*1}	22	*Eucalyptus grandis* A
8	*Acacia mangium* A	23	*Eucalyptus grandis* B
9	*Acacia mangium* B	24	*Eucalyptus grandis* C
10	*Acacia mangium* C-1^{*2}	25	*Eucalyptus hybrid* ^{*3}
11	*Acacia mangium* C-2^{*2}	26	*Eucalyptus nitens*
12	*Acacia mangium* D	27	*Eucalyptus saligna*
13	*Acacia mangium* E	28	*Eucalyptus urophylla*
14	*Acacia mangium* F		
15	*Acacia meransii*		

Same species with different letter names (A to F) are from different growing areas. ^{*1}: Hybrid of *Acacia mangium* and *A. auriculiformis* from different mother trees. ^{*2}: Same species from the same plantation area, but C-1 is 8 years old and C-2 is 12 years old. ^{*3}: Hybrid of *Eucalyptus camaldulensis* and *E. deglupta*.

Characteristics of reaction wood lignin in the various woods

A wood disk containing reaction wood portions was taken from a gymnosperm (*Pinus merkusii* Jungh. et de Vriese) and four angiosperms (*Paraserianthes falcataria* (L.) Nielses, *Melia azedarach* L., *Eusideroxylon zwageri* T. et B., *Avicennia sp.*). Three wood disk samples were collected for the leaning stem of *P. merkusii* and *P. falcataria*. When the expanded area on the disk was originated from the lower side of the stems or branches, the reaction wood portion was defined as compression wood in this thesis. On the other hand, when the expanded area was located on the upper side, it was defined as tension wood. Small wood blocks were cut from each wood disc at the several positions along the periphery of the discs for all the stems. Wood blocks were also cut at two positions at lower and upper side of the branch. Lignin content, aromatic ring type, and diastereomeric forms for the β-O-4 structure were determined by Klason method, nitrobenzene oxidation, and ozonation, respectively.

Characteristics of syringyl-guaiacyl lignin in the reaction wood in gymnosperm *Gnetum gnemon*

Samples were collected from a *G. gnemon* tree with a leaning stem in Bogor, Indonesia. A disk of wood (22 cm in diameter), a branch (8 cm in diameter), a lignified root (4 cm in diameter), bark and leaf samples were collected. Blocks of wood were cut from the sapwood at six positions along the periphery of the disk. The eccentric thickening growth was observed on the lower side of the disk, indicating that this side contained reaction wood that was comprised of compression wood. The Klason lignin content was determined, and the cell wall samples were subjected to nitrobenzene oxidation, ozonation, neutral sugar analysis. The peripheral positions 0° (= 360°) and 180° are the lower and upper sides of the leaning stem, respectively.

Results and discussion

Characteristics of lignin in Acacia and Eucalyptus woods

Structural diversities of *Acacia* and *Eucalyptus* lignins were evidenced by the wide ranges of variation in the experiment data, which was comparable to those obtained from different genera and families reported in the previous study (Akiyama et al. 2005). The experiment data from these 28 woods was categorized in two clearly distinguished groups on the basis of the syringyl ratio. Clear negative correlations were newly found between the syringyl ratio and lignin content, and between *erythro* ratios and lignin content within each genus (Figure 1 & 2). The syringyl ratio correlated positively with the *erythro* ratio among all samples regardless of the genus (Figure 3). The existence of a clear correlation between the syringyl and *erythro* ratios supports the hypothesis that the aromatic ring type is a decisive factor for controlling the diastereomeric forms of the β-O-4 structure. Alkali pulping experiments revealed that lignin is more easily removed when its syringyl ratio is higher (Figure 4). This result can be explained by the high reactivity of lignin with high syringyl and *erythro* ratios. Shimizu et al. quantitatively demonstrated using lignin model experiments that both the aromatic ring types (syringyl or guaiacyl) and β-O-4 diastereomeric type (*erythro* or *threo*) greatly affect the alkaline cleavage of the β-O-4 linkage during chemical pulping.[4,5]

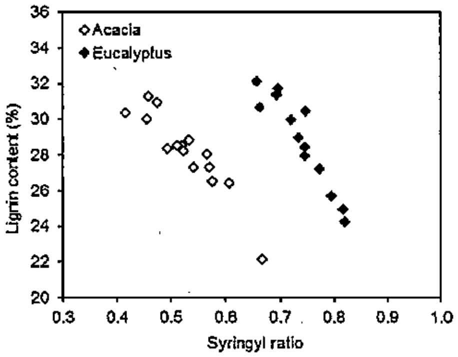

Figure 1. Correlation between syringyl ratio and lignin content

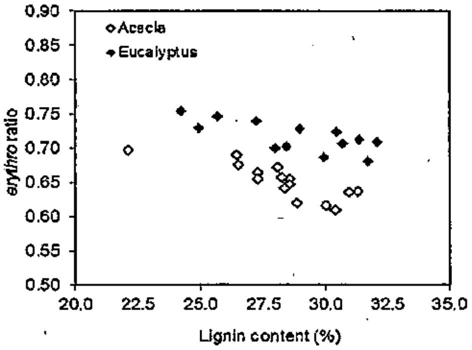

Figure 2. Correlation between lignin content and *erythro* ratio

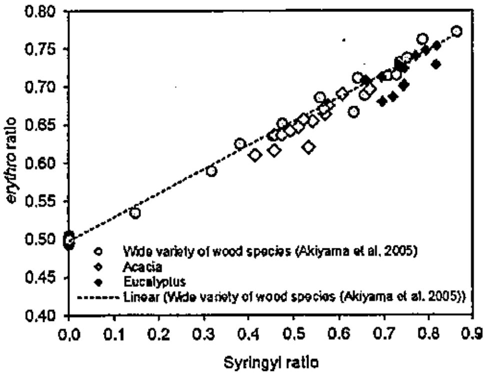

Figure 3. Correlation between syringyl ratio and *erythro* ratio

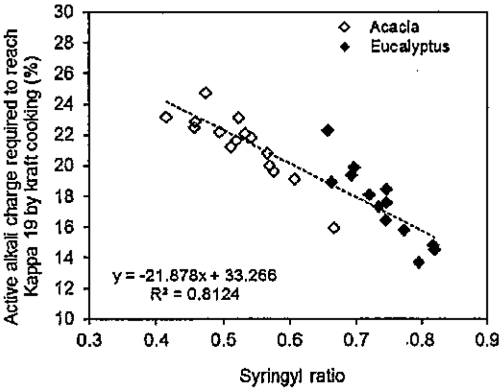

Figure 4. Correlation between syringyl ratio and pulpability

Characteristics of reaction wood lignin in the various woods

The reaction woods of five species examined were categorized to three-types based on the eccentric thickening growth and the lignin structures; a compression wood containing guaiacyl-*p*-hydroxyphenyl lignin typical for gymnosperms (GH-lignin-CW), a tension wood containing guaiacyl-syringyl lignin typical for angiosperms (GS-lignin-TW), and a compression wood exceptionally containing GS-lignin (GS-lignin-CW). Gymnosperm *Pinus merkusii* formed a typical compression wood of gymnosperm (GH-lignin-CW type). The lignin content increased from the upper side to lower side (compression wood part), and it was accompanied by the increase of proportion of *p*-hydroxyphenyl unit (H-ratio) and *erythro* ratio of β-O-4 structure. A positive correlation was found between these two ratios, suggesting that H-unit is a factor influencing on the *erythro* selective formation during lignin biosynthesis of gymnosperm.

Angiosperm *Eusideroxylon zwageri* contains guaiacyl-syringyl lignin, but was found to form an eccentric thickening growth on the lower side of the branch similarly to gymnosperm compression wood (GS-lignin-CW type). The upper side exhibited a less lignin, a higher syringyl ratio, and a higher *erythro* ratio of β-O-4 structure than the lower side. These tendencies found in GS-lignin-CW were similar to those found in the tension wood lignin of angiosperm (GS-lignin-TW type: *Paraserianthes falcataria, Melia azedarach,* and *Avicennia* sp.). .These result implied that, during their lignification, the reaction woods containing GS-lignin changes their chemical structures in a similar way in response to longitudinal growth stress, regardless of whether a wood forms eccentric growth on the compression or tension wood side. The syringyl ratio was correlated with the *erythro* ratio in all the reaction woods composing of guaiacyl-syringyl lignin, i.e. both for GS-lignin-CW and GS-lignin-TW types (Figure 5). In addition, lignin content showed clear negative correlations with syringyl and *erythro* ratios (Figure 6).

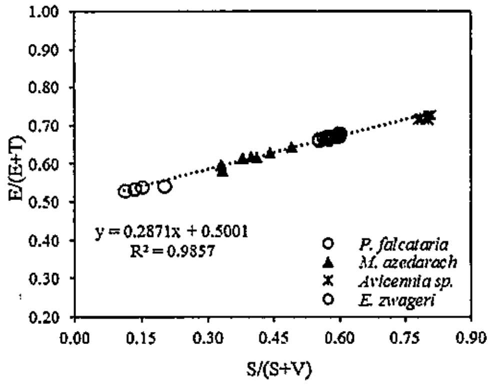

Figure 5 Correlation between syringyl ratio and *erythro* ratio

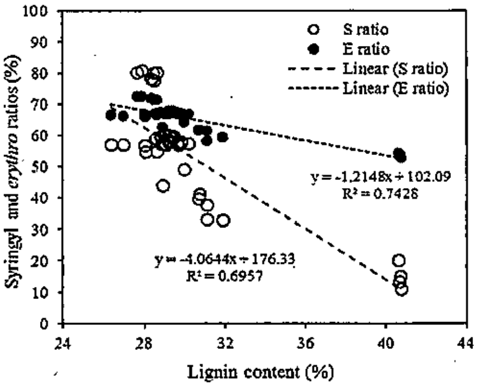

Figure 6 Correlation between lignin content and syringyl ratio

The leaning stem of *Gnetum gnemon* was wider on the lower side than on the upper side, similar to the case for compression wood in gymnosperms. The upper side contained less lignin and its lignin had a higher syringyl ratio and a higher *erythro* ratio of *β*-O-4 structure than the lower side (Figure 6). Therefore, the reaction wood lignin of this gymnosperm species was found to be GS-lignin-CW type as well as that of angiosperm *E. zwageri*. The H-units were minor components, but was richer in the lower side, which resembles the distribution in compression wood. The distribution of hemicellulose was found to be different from both typical tension wood and compression wood. A large part of the decreases in the glucose and xylose yields in the lower side wood was offset by increases in the mannose yield and lignin content. It was suggested that glucomannan rather than xylan is an important component of *G. gnemon* reaction wood. Furthermore, the *erythro* ratios of the *β*-O-4 structures in the leaf, bark, stem, branch, and root samples varied widely, and closely matched the distribution of the syringyl ratio.

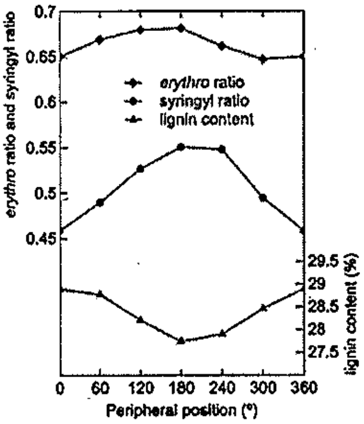

Figure 6. The distribution of *erythro* ratio, syringyl ratio, and lignin content in the reaction wood stem of *G. gnemon* lignins.

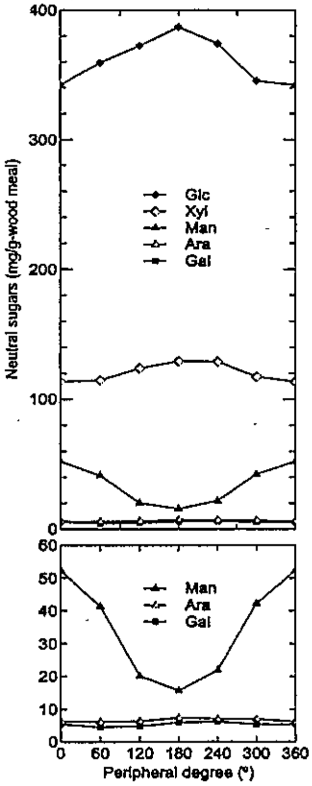

Figure 7. Sugar yields obtained from the reaction wood stem of *G. gnemon* by neutral sugar analysis.

References

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