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ORIGINAL ARTICLE

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# Distribution of lignin interunit bonds in the differentiating xylem of compression and normal woods of *Pinus thunbergii*

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**Abstract** The lignification process and lignin distribution at different stages of cell wall differentiation in the secondary xylem of compression and normal woods of *Pinus thunbergii* were investigated by thioacidolysis and subsequent desulfuration. We prepared 50-$\mu$m-thick, contiguous tangential sections of pine shoots, cut from the cambial zone through to mature xylem. In compression wood, uncondensed guaiacyl (G) and *p*-hydroxyphenyl (H) lignins were deposited simultaneously from early to late stages of lignification. The various types of G-G, G-H, and H-H dimers were detected in compression wood, and the ratio of G-H and H-H dimers to total dimers increased as lignification proceeded. In contrast, uncondensed and condensed H units were detected in trace amounts in normal wood. Significant differences in the relative distributions of lignin interunit linkages were not observed between compression and normal woods or between differentiating and mature xylems in either compression or normal woods.

**Key words** Compression wood · Lignification · Cell wall differentiation · Thioacidolysis · *p*-Hydroxyphenyl lignin

## Introduction

Compression wood is formed on the lower side of inclined stems or shoots of conifers, where dark-red areas of eccentric radial growth are observed. Morphological and chemical characteristics of compression wood are appreciably different from those of normal wood, as discussed in detail by Timell;[1] for example, as compared with normal wood, compression wood displays rounded tracheid cells, large intercellular spaces, the absence of an $S_3$ layer (inner layer of the secondary wall), and thick secondary walls containing more lignin and less cellulose. In addition, compression wood lignin has a higher proportion of *p*-hydroxyphenyl (H) units [e.g., about 18% H units of guaiacyl (G) units in compression wood of *Pinus thunbergii*][2] than does typical conifer lignin of normal wood, which contains H units in trace amounts.

The period of most active lignification is also considered to differ between compression and normal woods. Lignification processes in compression and normal woods have been studied by microautoradiography using radiolabeled precursors.[2–7] Terashima et al.[8] reported that lignin deposition is preceded by the deposition of polysaccharides and proceeds in three distinct stages: lignification of the cell corner and middle lamella just before $S_1$ formation, slow lignification during secondary wall formation, and the main lignification of the secondary wall just after the start of $S_3$ formation. Furthermore, Fukushima and Terashima[2] observed the following features of the lignification patterns in *Pinus thunbergii*, based on the incorporation of labeled precursors of *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) lignins:

1. Lignin is deposited in the same order in both compression and normal woods, i.e., H, G, and then S units.
2. G units are incorporated into the secondary wall of normal wood over a short period, just after the start of $S_3$ formation, while H units are mainly incorporated into the compound middle lamella (CML). In contrast, in compression wood, the incorporation of G units occurs over a long time, between the formation of the outer and inner $S_2$ layers, and H units are mainly incorporated into the secondary wall in differentiating xylem.
3. In normal wood, the occurrence of condensed G units are frequent in the CML and are low in the secondary wall, while in compression wood, condensed G units are homogenously distributed in all morphological regions. Recently, monomeric, dimeric, and trimeric thioacidolysis products containing G and H units were reported in spruce compression wood, suggesting that

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most of the H units were present in noncondensed structures.[9]

In this article, we clarify the heterogeneous formation of lignin interunit bonding in differentiating and mature xylems of compression and normal woods of pine. We used a chemical degradative method, thioacidolysis, to efficiently cleave arylglycerol-$\beta$-aryl ether bonds in lignins, followed by desulfuration over Raney nickel, to obtain information about the dimers (G-G, G-H, and H-H types).

## Materials and methods

### Materials

Three-year-old shoots (upright, 2.5 cm in diameter) and 4-year-old shoots (inclined, 2.5 cm in diameter) were harvested from *Pinus thunbergii* growing on the campus of Nagoya University in June, 1997. The bark was removed from each shoot, the blocks of shoots were frozen and fixed with ice on the freezing stage of a sliding microtome, and 50-$\mu$m-thick tangential sections (three sheets of 0.5 × 2.5 cm) were cut successively from the cambial zone to the mature xylem. The sections were numbered in order, beginning nearest to the cambium (No. 1), such that the degree of lignification became greater as the section number increased. The first and second sections (Nos. 1 and 2) were composed of compound middle lamella lignin, and the 13th and 14th sections (Nos. 13 and 14) were mature xylem in which lignification was complete.

### Chemical analysis

Thioacidolysis and subsequent Raney nickel desulfuration were performed according to the method previously described.[10–12] Section Nos. 1–10 were subjected to thioacidolysis, and Nos. 13 and 14 were combined and halved for analysis, represented by "No. 13" in this report. Section Nos. 11 and 12 were not used. Desulfuration of the thioacidolysis products of section Nos. 1–4, 10, and 13 from compression and normal woods was performed.

### Gas chromatography and gas chromatography-mass spectrometry analyses

The silylated derivatives were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). GC was performed using a 0.25 mm i.d. ×60 m TC-1 column (GL Science, Japan) with flame ionization detection (FID) and using $N_2$ as a carrier gas (200 kPa). The sample (3 $\mu$l) was injected with a moving-needle type injector at 250°C, and the temperature was programmed to increase from 180° to 280°C at 2°C/min. The mass spectra were recorded at 70 eV with an M-station JMS700 magnetic-sector mass spectrometer (JEOL, Japan), combined with a model 6890 gas chromatograph with a silica capillary column (30 m × 0.32 mm i.d., DB-1, Hewlett Packard, USA). The carrier gas was He, and the temperature was programmed to increase from 180° to 280°C at 2°C/min. All G-G dimers were confirmed by comparison with references previously reported,[11] and G-H and H-H dimers were tentatively assigned based only on their mass fragmentation patterns. The monomeric and dimeric products were quantified by GC, as previously described.[10,11]

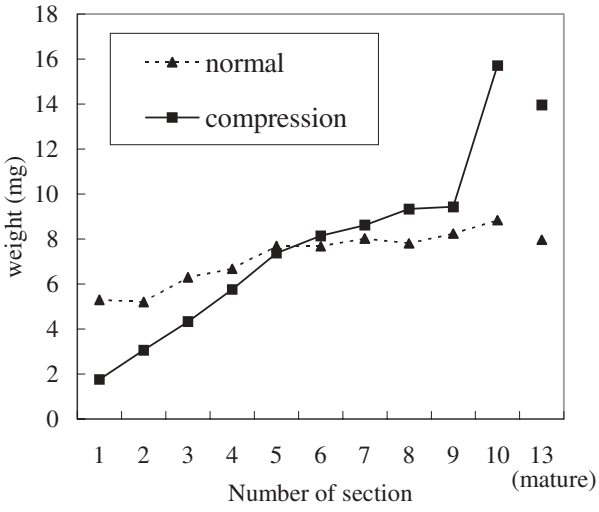


**Fig. 1.** Weights of sections from normal and compression woods. Section No. 13 is mature xylem

## Results and discussion

### Thioacidolysis monomers

Considering the weight of each section (Fig. 1), lignification should be complete at about section No. 10 in both compression and normal woods, and therefore, section No. 13 was analyzed as the mature xylem. Figure 2 shows the yields of thioacidolysis monomers derived from normal and compression woods. Yields were expressed as micromoles of product per section (per each volume) to estimate clearly the degree of lignin deposition per single cell. Expressing the yield as the product amount per gram would provide the estimation about the greater number of cells at the early stage of differentiating xylem where the weights of cells are lower than at the late stage. Thus, in this report, the yields cannot be compared precisely between normal and compression woods because the yields are not expressed as per gram of the section.

As the section numbers increased from the early to late stages of lignification, G monomers linked by $\beta$-aryl ether bonds increased in both normal and compression woods (Fig. 2). In normal wood, thioacidolysis H monomer peaks were detected in trace amounts, but the peaks overlapped with an unknown peak on the total ion chromatogram (TIC) and were therefore not quantified. Syringyl units were not detected in either normal or compression wood. Figure 2 shows that the rate of lignification differs significantly between normal and compression woods. In addition, the yield of G monomers from section No. 1 of normal

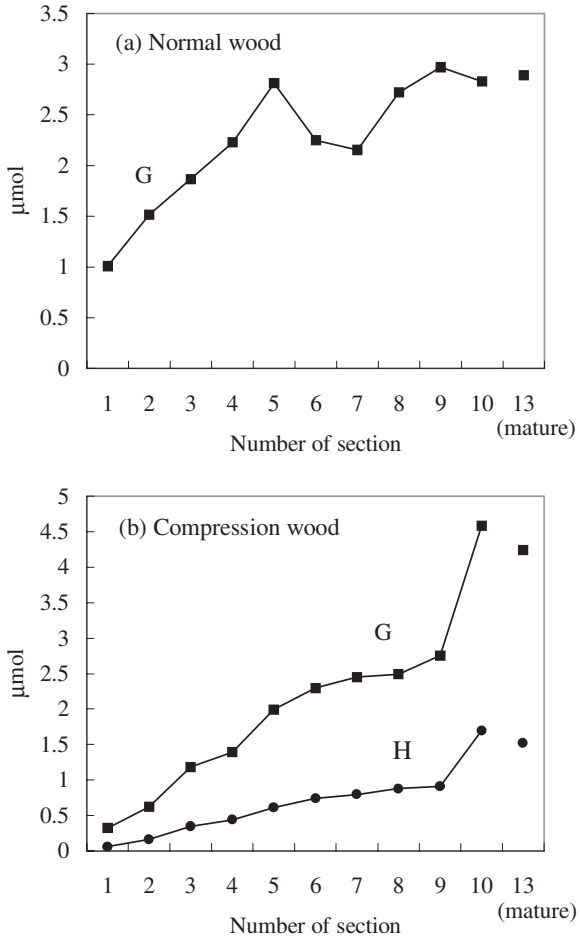


**Fig. 2.** Yields of thioacidolysis monomers obtained from normal (**a**) and compression (**b**) woods. *G*, guaiacyl monomers; *H*, *p*-hydroxyphenyl monomers

wood was about one third of that of section No. 13; however, in compression wood, the yield of G monomers from section No. 1 was only about one ninth of that of section No. 13, and the increase in the amount of lignin was perfectly reflected in the increase in the weights of the sections (Fig. 1). This result indicates that the amount of lignin present at the start of deposition, probably CML lignin, is higher in normal wood than in compression wood.

In compression wood, it was shown that G and H monomers were deposited simultaneously (Fig. 2b); however, the H/G ratio increased as lignification proceeded (Fig. 3). This suggests that more H lignin is deposited at a late stage of secondary wall formation in compression wood and is incorporated for a longer period of time, which is in agreement with the data obtained by autoradiography.[2]

## Thioacidolysis dimers

Figure 4 shows the TIC from the GC-MS analyses of trimethylsilylated thioacidolysis products after desulfuration

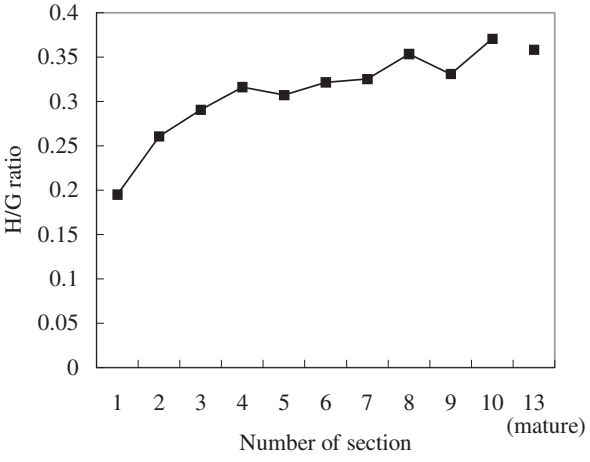


**Fig. 3.** The H/G ratios of thioacidolysis monomers obtained from compression wood

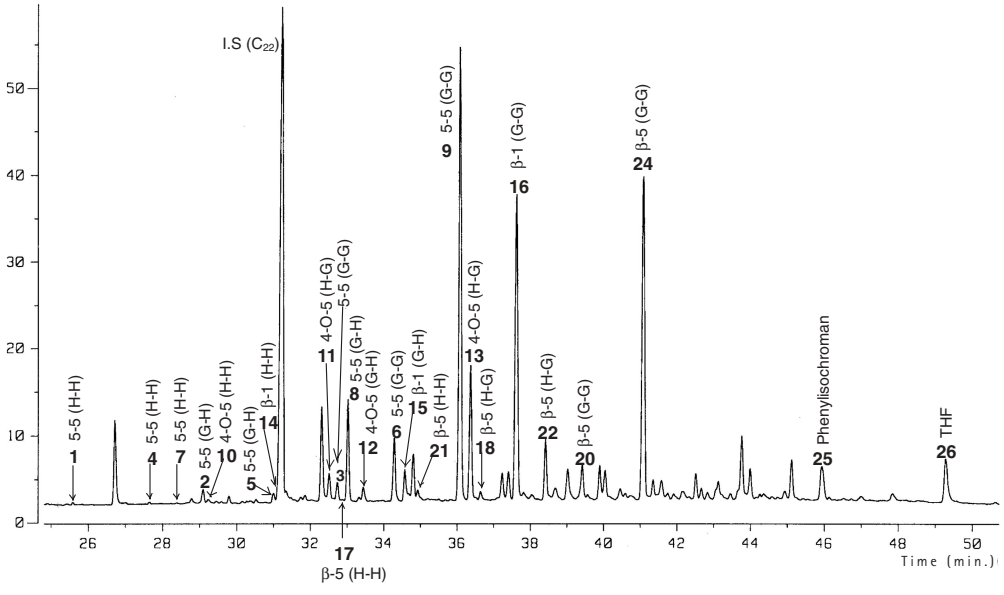


**Fig. 4.** Total ion chromatogram of trimethylsilylated thioacidolysis products after desulfuration over Raney nickel from the compression wood of pine. Peak assignments correspond to trimethylsilylated derivatives of the products given in Fig. 5. *I.S*, internal standard (docosane)

over Raney nickel. The structures of the compounds represented by the main peaks are shown in Fig. 5, and all mass spectral data are summarized in Table 1. The peak assignments of the main G-G dimers were based on both the mass spectral and elution order data previously described.[11,12] The assignments of G-H and H-H dimers were made by comparison with the fragmentation patterns of their corresponding G-G dimers. The occurrence of G-H and H-H dimers upon thioacidolysis of compression wood,[13] grass species,[13] suspension cultures of *Picea abies*,[14] and dehydrogenation polymers (DHP)[15] have been reported earlier; recently, dimers and trimers containing G and H units have been reported from spruce compression wood.[9] However, to our knowledge, the quantification of G-H and H-H dimers has not been described in detail, and in this report we further investigate the distribution of G-H and H-H thioacidolysis dimers in compression and normal woods.

To clarify the differences in lignin distribution of dimers between differentiating and mature xylems, six sections, Nos. 1–4, 10, and 13, were used for dimer analysis. Section No. 1 was the nearest to the cambium, and No. 13 was composed of mature xylem. The main dimers recovered from compression and normal woods are representative of the 5-5 (dimers **1**–**9**), 4-*O*-5 (dimers **10**–**13**), *β*-1 (dimers **14**–

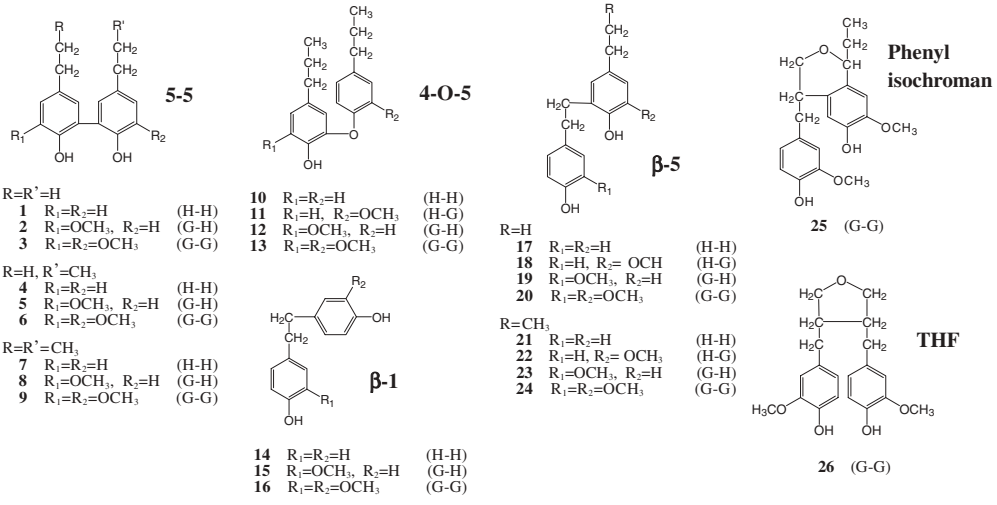


**Fig. 5.** Main dimers obtained from compression and normal woods after thioacidolysis and subsequent desulfuration. All mass spectral data of the compounds are listed in Table 1. Compounds **19** and **23** were not detected, as discussed in the text. *THF*, tetrahydrofuran

**Table 1.** Mass spectral data of trimethylsilylated thioacidolysis products from compression and normal woods after desulfuration over Raney nickel

| Compound | | $M^+$ | Fragments | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5-5 | | | | | | | | | | | |
| **1** | H-H | 386 (67) | 371 (8) | 357 (59) | 147 (8) | 73 (100) | | | | | |
| **2** | G-H | 416 (72) | 401 (18) | 387 (33) | 386 (15) | 147 (9) | 73 (100) | | | | |
| **3** | G-G | 446 (95) | 431 (34) | 417 (30) | 416 (30) | 147 (5) | 73 (700) | | | | |
| **4** | H-H | 400 (75) | 385 (11) | 371 (56) | 147 (8) | 73 (100) | | | | | |
| **5** | G-H | 430 (71) | 415 (19) | 401 (28) | 400 (14) | 147 (10) | 73 (100) | | | | |
| **6** | G-G | 460 (94) | 445 (34) | 431 (25) | 430 (27) | 147 (5) | 73 (100) | | | | |
| **7** | H-H | 414 (86) | 399 (8) | 385 (79) | 147 (9) | 73 (100) | | | | | |
| **8** | G-H | 444 (93) | 429 (21) | 415 (42) | 414 (13) | 147 (10) | 73 (100) | | | | |
| **9** | G-G | 474 (100) | 459 (40) | 445 (45) | 444 (28) | 147 (6) | 73 (100) | | | | |
| 4-*O*-5 | | | | | | | | | | | |
| **10** | H-H | 342 (95) | 427 (66) | 313 (100) | 179 (22) | 73 (41) | | | | | |
| **11** | H-G | 372 (100) | 357 (46) | 343 (75) | 327 (16) | 313 (8) | 179 (28) | 73 (41) | | | |
| **12** | G-H | 372 (100) | 357 (38) | 343 (58) | 342 (49) | 313 (26) | 209 (14) | 73 (46) | | | |
| **13** | G-G | 402 (100) | 387 (34) | 373 (43) | 372 (25) | 357 (6) | 343 (15) | 209 (8) | 179 (9) | 73 (30) | |
| *β*-1 | | | | | | | | | | | |
| **14** | H-H | 358 (13) | 343 (4) | 179 (100) | 73 (74) | | | | | | |
| **15** | G-H | 388 (18) | 373 (7) | 209 (100) | 179 (48) | 73 (73) | | | | | |
| **16** | G-G | 418 (35) | 403 (7) | 209 (100) | 179 (34) | 73 (79) | | | | | |
| *β*-5 | | | | | | | | | | | |
| **17** | H-H | 386 (17) | 371 (5) | 207 (29) | 179 (100) | 73 (22) | | | | | |
| **18** | H-G | 416 (30) | 401 (7) | 237 (40) | 222 (12) | 207 (12) | 179 (100) | 73 (37) | | | |
| **20** | G-G | 446 (20) | 431 (3) | 237 (14) | 222 (6) | 209 (100) | 207 (12) | 193 (6) | 179 (10) | 73 (40) | |
| **21** | H-H | 400 (15) | 385 (6) | 221 (39) | 179 (100) | 73 (36) | | | | | |
| **22** | H-G | 430 (32) | 415 (7) | 251 (50) | 236 (6) | 221 (6) | 207 (20) | 193 (5) | 179 (100) | 73 (43) | |
| **24** | G-G | 460 (25) | 445 (4) | 251 (16) | 236 (3) | 221 (3) | 209 (100) | 207 (12) | 193 (5) | 179 (9) | 73 (29) |

Numbers in parentheses show relative abundance. Mass spectra of compounds **25** and **26** were reported previously in the literature[11]
M, molecular weight; H, *p*-hydroxyphenyl unit; G, guaiacyl unit

**Table 2.** The relative distribution (mol%) and total yield of dimers recovered from thioacidolysis and subsequent desufuration of normal and compression woods

| | Normal wood | | | | | | Compression wood | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. 1 | No. 2 | No. 3 | No. 4 | No. 10 | No. 13 | No. 1 | No. 2 | No. 3 | No. 4 | No. 10 | No. 13 |
| 5-5 | | | | | | | | | | | | |
| G-G | 32.7 | 32.1 | 34.3 | 33.2 | 26.3 | 35.7 | 26.3 | 33.8 | 32.2 | 32.8 | 27.2 | 30.1 |
| G-H | 0.8 | 0.5 | 0.4 | 0.5 | 0.5 | 0.4 | 3.7 | 5.0 | 4.1 | 7.7 | 6.5 | 6.6 |
| H-H | nd | nd | nd | nd | nd | nd | tr | 0.2 | 0.2 | 0.2 | 0.4 | 0.5 |
| Total | 33.4 | 32.6 | 34.8 | 33.7 | 26.8 | 36.1 | 30.1 | 39.0 | 36.4 | 40.6 | 34.2 | 37.1 |
| $\beta$-5 | | | | | | | | | | | | |
| G-G | 30.3 | 29.8 | 30.5 | 29.3 | 34.0 | 26.8 | 21.6 | 19.8 | 23.9 | 18.1 | 19.6 | 20.0 |
| G-H | 0.4 | 0.3 | 0.3 | 0.5 | 0.3 | 0.3 | 1.6 | 2.4 | 2.2 | 3.6 | 3.8 | 4.0 |
| H-H | nd | nd | nd | nd | nd | nd | tr | tr | tr | tr | 0.2 | 0.4 |
| Total | 30.7 | 30.2 | 30.8 | 29.8 | 34.3 | 27.0 | 23.1 | 22.2 | 26.1 | 21.7 | 23.6 | 24.4 |
| 4-*O*-5 | | | | | | | | | | | | |
| G-G | 8.3 | 7.3 | 6.1 | 9.1 | 7.1 | 7.8 | 3.9 | 8.3 | 7.5 | 8.2 | 8.2 | 8.5 |
| G-H | nd | tr | tr | tr | tr | tr | 1.3 | 1.2 | 1.5 | 2.9 | 2.8 | 2.9 |
| H-H | nd | nd | nd | nd | nd | nd | nd | nd | nd | tr | 0.2 | 0.2 |
| Total | 8.3 | 7.3 | 6.1 | 9.1 | 7.1 | 7.8 | 5.3 | 9.5 | 8.9 | 11.1 | 11.2 | 11.5 |
| $\beta$-1 | | | | | | | | | | | | |
| G-G | 27.1 | 27.6 | 27.0 | 24.6 | 30.1 | 26.0 | 31.2 | 21.8 | 22.0 | 19.8 | 19.7 | 21.5 |
| G-H | nd | nd | nd | tr | tr | tr | 1.9 | 2.1 | 1.8 | 1.7 | 2.2 | 2.1 |
| H-H | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | tr | tr |
| Total | 27.1 | 27.6 | 27.0 | 24.6 | 30.1 | 26.0 | 33.1 | 23.9 | 23.9 | 21.5 | 21.9 | 23.6 |
| THF | | | | | | | | | | | | |
| G-G | 0.5 | 2.3 | 1.4 | 2.8 | 1.7 | 3.1 | 8.4 | 5.4 | 4.8 | 5.0 | 9.1 | 3.3 |
| Total (%) | | | | | | | | | | | | |
| G-G | 98.9 | 99.1 | 99.3 | 99.0 | 99.2 | 99.3 | 91.5 | 89.1 | 90.3 | 84.0 | 83.9 | 83.4 |
| G-H | 1.1 | 0.9 | 0.7 | 1.0 | 0.8 | 0.7 | 8.5 | 10.7 | 9.5 | 15.9 | 15.3 | 15.6 |
| H-H | – | – | – | – | – | – | 0.0 | 0.2 | 0.2 | 0.2 | 0.8 | 1.0 |
| Total yield of dimers ($\mu$mol) | 0.21 | 0.27 | 0.35 | 0.36 | 0.61 | 0.62 | 0.07 | 0.15 | 0.39 | 0.37 | 1.32 | 1.23 |

Yields are expressed as micromoles per section
THF, tetrahydrofuran; tr, trace; nd, not detected

**16**), $\beta$-5 (dimers **17**–**24**), phenylisochroman (dimer **25**), and tetrahydrofuran (THF) (dimer **26**) bonding patterns, as presented in Table 2. Among dimers of G-H and H-H, dimers **8**, **10**–**12**, **15**, **20**, and **21** have been previously identified as acetylated derivatives[9] and the others are first reported here. The 5-5 and $\beta$-5 linkages were dominant in both compression and normal woods, and the relative distribution of those dimers did not differ significantly between early and late stages of lignification. The relative distribution of the 4-*O*-5 linkage was comparatively higher in compression wood than in normal wood, and this linkage showed a tendency to increase as lignification proceeded. The distribution of the THF dimer was relatively higher in compression wood than in normal wood. The peak of the phenylisochroman linkage (dimer **25**) overlapped with an unknown peak on the chromatogram and was not determined quantitatively. This phenylisochroman thioacidolysis dimer contains $\beta$-6 linkages, but it was suggested[16] that this structure is an analogous sidechain-shifted isomer of the unique $\beta$-1 structure with a six-membered di-$\alpha$-ether ring. The G-G dimer derived from the pinoresinol linkage,[12] which is a minor structure in softwood lignin, was recovered in trace quantities from all samples (data not shown). The distribution of interunit bonding patterns of compression wood did not differ significantly from that of normal wood. Significant differences in the distributions of dimers were also not confirmed between differentiating and mature xylems in either compression or normal woods.

In addition to G-G dimers, G-H dimers were detected from both normal and compression woods, but H-H dimers were detected only from compression wood (Table 2). In both normal and compression woods, the 5-5 and $\beta$-5 structures were prominent in G-H and H-H types, as well as in G-G dimers. In terms of dimers between G and H, dimers with $\beta$-5 linkages, compounds **18** and **22**, were detected, whereas compounds **19** and **23** were not detected, suggesting the former are the preferential forms for the $\beta$-5 bonding pattern. The following relative frequencies of bonding patterns were reported among the thioacidolysis products from dehydrogenation polymers (DHP):[15]

1. For DHPs obtained from coniferyl and *p*-coumaryl alcohol prepared by end-wise polymerization: $\beta$-5 (42%), 5-5 (37%), $\beta$-1 (10%), $\beta$-$\beta$ (8%) and 4-*O*-5 (3%) dimers; prepared by bulk polymerization: $\beta$-5 (53%), 5-5 (21%), $\beta$-1 (2%), $\beta$-$\beta$ (24%) and 4-*O*-5 (0%) dimers.
2. For DHPs from only *p*-coumaryl alcohol prepared by end-wise polymerization: $\beta$-5 (56%) and 5-5 (44%) dimers.

Compared with the dimers of these DHPs, the relative distributions of dimers in pine compression wood and normal wood lignins were more similar to that of DHP prepared by

end-wise polymerization, as shown in Table 2. H-H dimers in compression wood gave a series of variable bonding patterns similar to those of G-G dimers, although 4-*O*-5 and $\beta$-1 types were not detected from DHP synthesized from *p*-coumaryl alcohol alone in vitro. This may be due to differences between polymerization in vitro and polymerization in the cell wall.

As shown in Table 2, the relative proportion of G-H and H-H dimers among all dimers in compression wood increased from the early to late stages (8.5% to 16.6%), while G-H dimers in normal wood were almost constant at around 1%. This result suggests that, in normal wood, H units are mainly incorporated into the CML, which supports the observations from the microautoradiographic study.[2] Several studies have shown that the CML lignin of normal wood has a significantly higher concentration and greater proportion of H units than does the secondary wall lignin.[17,18] It was also reported that H units located in the CML of normal wood are rich in condensed structures.[8]

*P*-Hydroxyphenyl units are considered to contribute to the formation of condensed units of lignin because H units have more conjugating sites at the 3- and 5-positions of the aromatic ring than do G units. However, in this study, the yield ratio of G-H and H-H dimers to H monomers (i.e., condensed to uncondensed units) did not differ significantly between the early and late stages in compression wood (data not shown). The investigation of DHPs upon thioacidolysis demonstrated that the copolymerization of H units with G units does not substantially decrease the dimer yield, and it has been proposed that H units do not exclusively participate in the more condensed linkages of lignin polymers, in which most of the uncondensed H units occur at the terminal position with free phenolic groups.[15] Dimers and trimers containing H units have also been detected in small amounts in spruce compression wood, where most of the H units appeared to be uncondensed $\beta$-*O*-4 structures.[9] The results of thioacidolysis presented here may support the assumption that the occurrence of more H units does not contribute to the presence of more condensed linkages.

We note that thioacidolysis only provides information about monomers and dimers that are linked with $\beta$-aryl ether bonds in lignin. It has been shown that about 50% of lignin can be characterized by thioacidolysis.[15] Therefore, the nature of all the lignin in the cell wall may not be represented by thioacidolysis. However, the results of thioacidolysis in this work agree well with most of the observations made by autoradiography using selective radiolabeled precursors.[2]

## Conclusions

The thioacidolysis monomer analysis of contiguous tangential sections in the differentiating xylem showed differences in the ligninfication process between compression and normal woods. The dimer analysis showed that the relative distributions of condensed linkages of G-G, G-H, and H-H dimers were not significantly different between normal and compression woods or between differentiating and mature xylems in either compression or normal woods.

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## References

1. Timell TE (1986) In: compression wood in gymnosperms vol 1. Springer, Berlin Heidelberg New York
2. Fukushima K, Terashima N (1991) Heterogeneity in formation of lignin part XV: formation and structure of lignin in compression wood of *Pinus thunbergii* studied by microautoradiography. Wood Sci Technol 25:371–381
3. Terashima N, Fukushima K (1988) Heterogeneity in formation of lignin-XI: an autoraciographic study of the heteroge neous formation and structure of pine lignin. Wood Sci Technol 22:259–270
4. Terashima N (1989) An improved radiotracer method for studying formation and structure of lignin. In: Lewis NG, Paice MG (eds) Plant cell wall polymers, biogenesis, and degradation. ACS Symposium Series 399. American Chemical Society, Washington, DC, pp 148–159
5. Takabe K, Fujita M, Saiki H (1985) Autoradiographic investigation of lignification in the cell walls of Cryptomeria (*Cryptomeria japonica* D. Don). Mokuzai Gakkaishi 31:613–619
6. Takabe K, Fujita M, Harada H, Saiki H (1981) Lignification process of Japanese black pine (*Pinus thunbergii* Parl.) trachieds (in Japanese). Mokuzai Gakkaishi 27:813–820
7. Fujita M, Harada H (1979) Autoradiographic investigations of cell wall development. II. Tritiated phenylalanine and ferulic acid assimilation in relation to lignification. Mokuzai Gakkaishi 25:89–94
8. Terashima N, Nakashima J, Takabe K (1996) Proposed structure for protolignin in plant cell walls. In: Lewis NG, Sarkanen S (eds) Lignin and lignan biosynthesis. ACS Symposium Series 697, American Chemical Society, Washington, DC, pp 181–193
9. Önnerud H (2003) Lignin stuctures in normal and compression wood. Evaluation by thioacidolysis using ethanethiol and methanethiol. Holzforschung 57:377–384
10. Rolando C, Monties B, Lapierre C (1992) In: Lin SY, Dence CW (eds) Methods in lignin chemistry. Springer, Berlin Heidelberg New York, pp 334–348
11. Lapierre C, Pollet B, Monties B (1991) Thioacidolysis of spruce lignin: GC-MS analysis of the main dimers recovered after Raney nickel desulphuration. Holzforschung 45:61–68
12. Lapierre C, Pollet B, Monties B (1991) Lignin structural fingerprint by thioacidolysis followed with Raney nickel desulphuration. International Symposium on Wood and Pulping Chemistry, Melbourne pp 543–549.
13. Lapierre C (1993) Application of new methods for investigation of lignin structure. In: Jung HG, Buxton DR, Hatfield DR, Ralph J (eds) Forage cell wall structure and digestibility. American Society of Agronomy, Madison, Wisconsin, USA, pp 133–166
14. Brunow G, Kilpelainen I, Lapierre C, Lundquist K, Simola KL, Lemmetyinen J (1993) The chemical structure of extracellular lignin released by cultures of *Picea abies*. Phytochemistry 32:845–850
15. Jacquet G, Pollet B, Lapierre C, Francesch C, Rolando C, Faix O (1997) Thioacidolysis of enzymatic dehydrogenation polymers from *p*-hydroxyphenyl, guaiacyl and syringyl precursors. Holzforschung 51:349–354
16. Peng J, Lu F, Ralph J (1999) Isochroman lignin trimers from DFRC-degraded *Pinus taeda*. Phytochemistry 50:659–666
17. Whiting P, Goring DAI (1982) Chemical characterization of tissue fractions from the middle lamella and secondary wall of black spruce tracheids. Wood Sci Technol 16:261–267
18. Whiting P, Favis BD, St-Germain FGT, Goring DAI (1981) Fractional separation of middle lamella and secondary wall tissue from spruce wood. J Wood Chem Technol 1:29–42