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Ratio of *erythro* and *threo* forms of β-O-4 structures in tension wood lignin☆

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Abstract

The ratio of *erythro* and *threo* forms of β-O-4 structures in tension wood lignin was investigated by ozonation analysis of wood meal taken from various positions in the stem of yellow poplar (*Liriodendron tulipifera*). The proportion of the *erythro* form was higher in tension wood than in opposite wood, and the methoxyl group content showed a similar trend. The proportion of the *erythro* form and the methoxyl group content in the 7 positions in the stem lignin was correlated (correlation coefficient $R = 0.98$), suggesting that the type of aromatic ring, syringyl or guaiacyl, is one of the factors which stereochemically controls the ratio of *erythro* and *threo* forms of β-O-4 structures during lignin formation.

Keywords: *Liriodendron tulipifera*; *Magnoliaceae*; Ozonation; *Erythro* and *threo*; Arylglycerol-β-aryl ether; Quinone methide; Syringyl and guaiacyl; Tension wood; Angiosperms; Hardwood; Lignin

1. Introduction

Arylglycerol-β-aryl ether type structures (β-O-4 structures) are predominant linkage types in lignin (Adler, 1977). This linkage type can either be in the *erythro* or *threo* forms since its three carbon side chain contains two asymmetric carbons. It has been reported that the *erythro* form of the β-O-4 structure predominates in angiosperms (Lundquist. 1979; Hauteville et al., 1986; Tollier et al., 1986; Matsumoto et al., 1993; Saake et al., 1996; Akiyama et al., 2002), while *erythro* and *threo* forms are present in about equal amounts in gymnosperms (Lundquist, 1980; Matsumoto et al., 1986).

The ratio of *erythro* and *threo* forms of β-O-4 structure is likely determined by how water addition to the quinone methide intermediates occurs (Fig. 1) (Adler, 1977; Nakatsubo et al., 1976; Brunow et al., 1993). The predominance of *erythro* form in angiosperms would presumably be caused by the different frequency of water addition to the possible two sites, *Re* and *Si* faces. Since participation of any enzyme has not been reported in the process of water addition, there has been interest in the factors controlling the proportion of *erythro* and *threo* forms. The aromatic ring type in lignin was suggested as a factor based on analyses of lignin chemical structure (Nimz et al., 1984; Bardet et al., 1986, 1998) and model experiments using lignin model compounds (Brunow et al., 1993). The effects of solvents and acidity were also indicated in the model experiments (Nakatsubo et al., 1976; Brunow et al., 1993). The two aromatic ring types, "syringyl" and "guaiacyl", are distinguished by the number of the methoxyl group substituents (Fig. 1). It may be possible to evaluate the effect of the methoxyl group substituents on the ratio of *erythro* and *threo* forms of β-O-4 structures by analyses of samples taken from different parts of the same stem with uneven distribution of syringyl/guaiacyl (S/G) ratio. Such heterogeneity of S/G ratio has been well known in tension wood (Bland, 1958; Musha and Goring, 1975; Lapierre and Monties, 1986; Baba et al., 1996; Aoyama et al., 2001; Donaldson, 2001). Tension wood is produced in the

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☆ Part three in the series "*Erythro*/*threo* ratio of β-O-4 structures as an important structural characteristic of lignin".

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upper side of a leaning woody angiosperm stem with eccentric thickening growth, and is characterized by gelatinous fiber, high growth stress, and less lignin content than its counterpart in the under side, which is called opposite wood.

In this study, the distributions of *erythro* and *threo* forms of β-O-4 structures and methoxyl group contents in lignin of Yellow Poplar (*Liriodendron tulipifera*) tension wood were investigated in order to elucidate the relationship between the aromatic ring types and the diastereomer ratio of the β-O-4 structure.

2. Results and discussion

2.1. Distribution of lignin content and syringyl and guaiacyl units

Wood meal samples prepared from specific positions along the periphery of the wood disk (Fig. 2) were subjected to lignin and methoxyl group content determinations. Lignin and methoxyl group contents in lignin of wood meals were plotted against their peripheral positions (Fig. 3a and b), to illustrate the uneven distribution of each chemical characteristic in the stem. The 0 (= 360) and 180 peripheral degrees represent the tension and opposite wood sides, respectively. The stem employed did not have gelatinous fibers, but the highest longitudinal growth stress has been confined in the upper side of the leaning stem (Okuyama et al., 1994). The lignin contents of the tension wood were lower than those of the opposite wood (Fig. 3a), as expected (Bland, 1958; Timell, 1969; Donaldson, 2001).

Methoxyl group analyses were performed for wood meal as well as for Klason lignin. When wood meal is used for such determinations, there is a possibility to overestimate the methoxyl content because of the presence of methoxyl groups and some unknown structures in carbohydrates (Goto et al., 2001). On the other hand, the determination of methoxyl group of Klason lignin minimizes such interference from carbohydrates but may underestimate the content because of the possible loss of methoxyl group from lignin during the Klason treatment. Nonetheless, the methoxyl group content determined for wood meal showed the same trend as that determined for Klason lignin (Fig. 3b).

In contrast to the lignin content distribution, the methoxyl group content in Klason lignin clearly decreased in the direction of tension to opposite wood (Fig. 3b). The decrease in the methoxyl group content must result from a decrease in the syringyl/guaiacyl ratio. It has been recognized that the tension wood is characterized by lower lignin content and higher syringyl/guaiacyl ratio than opposite wood (Bland, 1958; Bland and Scurfield, 1964; Musha and Goring, 1975; Aoyama et al., 2001). Some studies reported the reverse trend or the absence of significant differences in those structural characteristics between tension and opposite woods (Lapierre and Monties, 1986; Baba et al., 1996).

2.2. Distribution of erythro and threo forms of β-O-4 structures

The ratio of *erythro* and *threo* forms of β-O-4 structures was obtained as the yield ratio of two ozonation products, erythronic and threonic acids (Akiyama et al.,

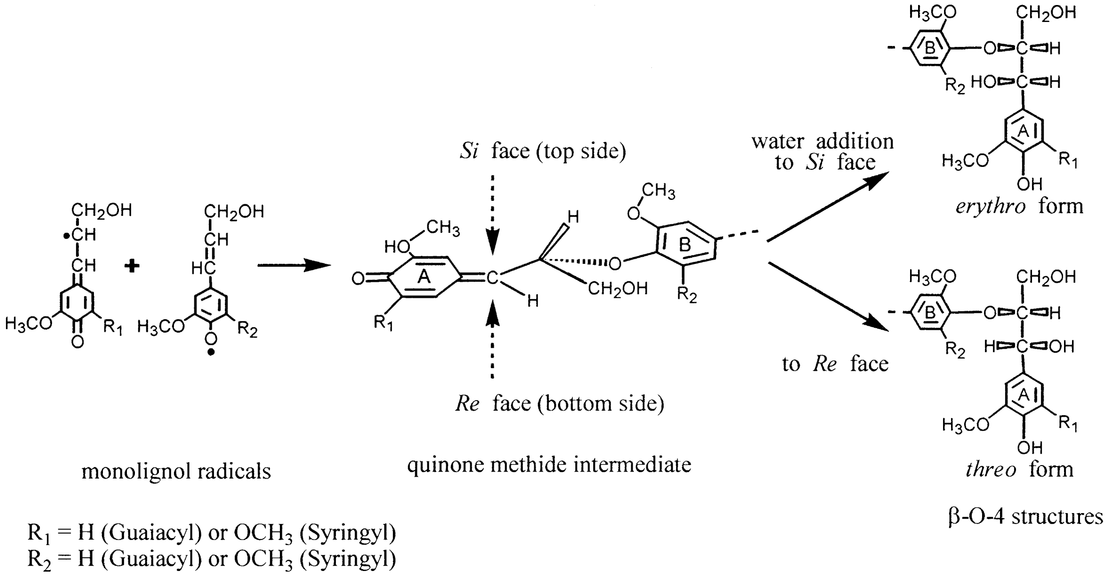

Fig. 1. Formation of *erythro* and *threo* forms of β-O-4 structures by the addition of water to the quinone methide intermediates. Only the *syn* isomer of the quinone methide intermediate ($R_1 = H$) was illustrated here, although this structure must contain *syn* and *anti* isomers.

2002). The reaction time of 2 h was used for the determination of the ratio because this ratio became almost constant thereafter.

Wood meal samples from 13 positions along the periphery of the wood disk were subjected to ozonation analysis to elucidate the distribution of *erythro* and *threo* forms of β-O-4 structures in the stem. As shown in Fig. 3c, the ratio of *erythro* form was as high as 76% in the tension wood at around 0 peripheral degree, in agreement with previous studies in which the *erythro* form of β-O-4 structure dominates in all angiosperms examined (Lundquist, 1979; Hauteville et al., 1986; Tollier et al., 1986; Matsumoto et al., 1993; Saake et al., 1996; Akiyama et al., 2002). The diastereomeric excess of *erythro* form of β-O-4 structure (over 50%) suggests significant stereoselective water addition to the Si face of quinone methide intermediates during lignin formation. Similar to the distribution of methoxyl group content, the ratio of *erythro* form in this stem decreased in the direction of tension to opposite wood, with the lowest value of 73% obtained at 180 peripheral degree (Fig. 3c). From this result, it can be concluded that the ratio of *erythro* and *threo* forms of β-O-4 structure is variable within a single stem with development of tension wood, although the observed change of the ratio as well as methoxyl content was not large.

The content of β-O-4 structures in lignin was evaluated as the total yield of erythronic and threonic acids for wood meals obtained from each peripheral position. As shown in Fig. 4, the total yields of these acids after 2 h ozonation of the wood meal at around 0 peripheral degree was obtained in a good yield which was 0.344 mmol per 1 gram wood meal and 0.3 per phenylpropane unit (Mw of 1 phenylpropane unit is assumed to be 200). The yields of these acids ranged from 0.26 to 0.33 per one phenylpropane unit depending on the peripheral position but no clear relationships between the yields of these acids and the peripheral position could be established in these experiments (data not shown).

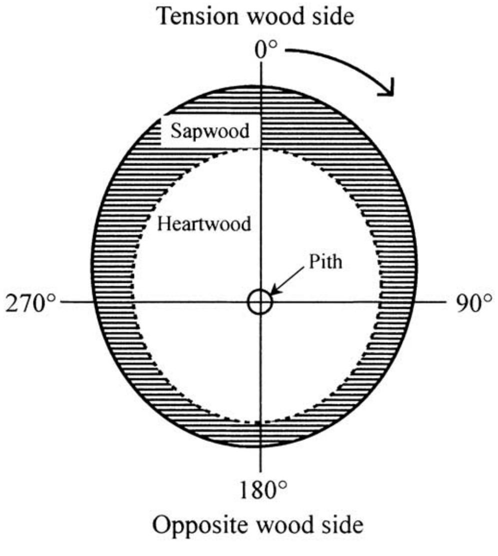

Fig. 2. The sampling zones from a cross-section of the leaning stem of yellow poplar (*Liriodendron tulipifera*). Small wood blocks consisting of 30–50 growth rings in sapwood were collected along the periphery of the stem.

2.3. Relationship between the ratio of erythro form and the methoxyl group content

In Fig. 5, the ratio of diastereomeric forms of β-O-4 structures at different peripheral positions determined by ozonation were plotted against methoxyl group contents in the corresponding Klason lignin and wood

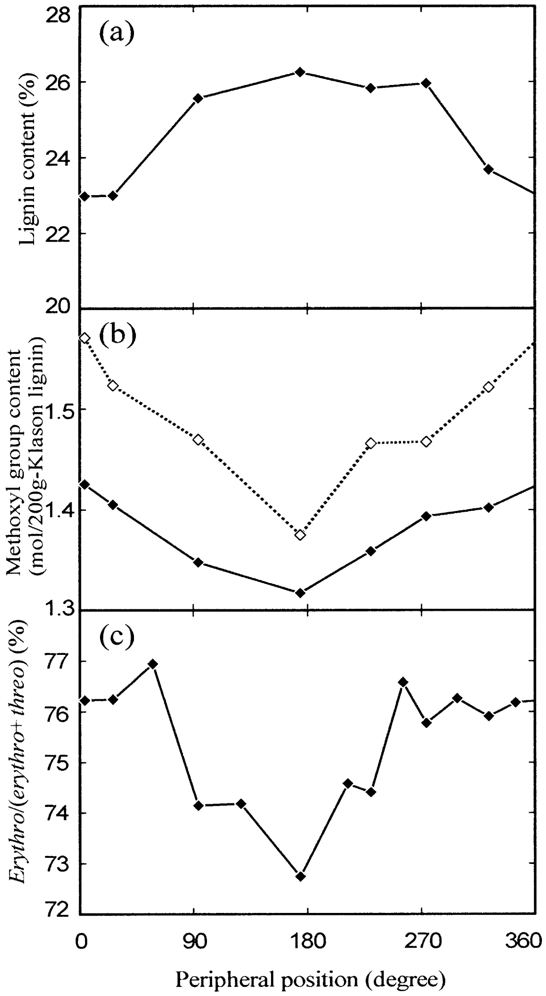

Fig. 3. Distributions of (a) lignin content, (b) methoxyl group content, and (c) the ratio of *erythro* form of β-O-4 structures along the periphery of the leaning stem of yellow poplar (*Liriodendron tulipifera*), 0° (360°) for tension wood side, 180° for opposite wood side. The peripheral degrees in this figure correspond to those in Fig. 2. (b) solid line: methoxyl group content determined for wood meal and expressed as content per lignin (Klason lignin + acid soluble lignin), dotted line: methoxyl group content determined for Klason lignin. (c) The ratio of *erythro* and *threo* forms of β-O-4 structures was determined by ozonation for 2 h.

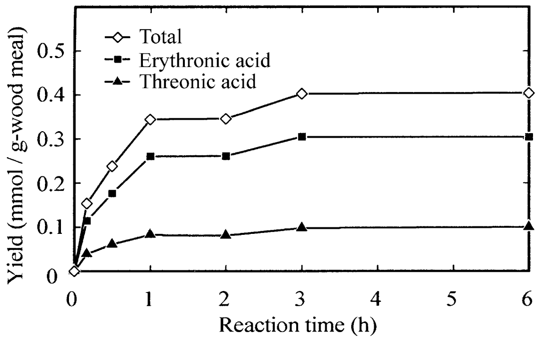

Fig. 4. Yields of erythronic and threonic acids obtained by ozonation of yellow poplar wood meal at 0 peripheral degree in tension wood.

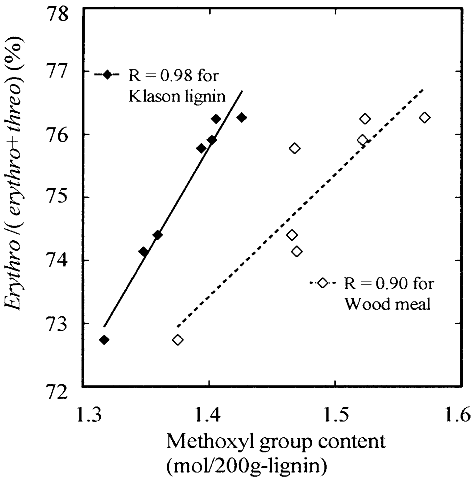

Fig. 5. Relationships between the proportion of *erythro* form in β-O-4 structures and methoxyl group contents.

meal. The methoxyl group contents determined for Klason lignins and for wood meal are highly correlated with the ratios of *erythro* form, fitting well the straight line (correlation coefficients, $R = 0.98$, 0.90, respectively).

These trends agree with previous NMR studies which demonstrated the relationship between the syringyl unit and the *erythro* form of β-O-4 structures (Nimz et al., 1984; Bardet et al., 1986, 1998). The ^{13}C NMR study of beech MWL (Nimz et al., 1984) and the ^{13}C NMR 2D-INADEQUATE spectrum of ^{13}C-enriched aspen MWL (Bardet et al., 1986, 1998) showed that the *erythro* form of β-syringyl ether structure predominates among the four linkage types (*erythro* and *threo* forms of β-syringyl ethers, and *erythro* and *threo* forms of β-guaiacyl ethers). These findings indicate that the type of aryl group (B-ring in Fig. 1) affects the ratio of *erythro* and *threo* forms, as previously demonstrated in a model experiments (Brunow et al., 1993). In this experiments quinone methide of the β-O-4 dimer model with syringyl unit (A-ring in Fig. 1) gave higher proportion of *erythro* form than the quinone methide with guaiacyl unit (Brunow et al., 1993). During actual lignin formation, however, the type of A-ring may also affect the ratio of *erythro* and *threo* forms of β-O-4 structures. The methoxyl group content determined here, containing the information of all methoxyl groups both in A- and B-rings of β-O-4 structures, will be suitable for evaluating the effects of the ring types, although the methoxyl groups of the other linkage types are also included in this method. The relationship observed in Fig. 5 supported the hypothesis that the type of aromatic ring, syringyl or guaiacyl, is one of the factors which determine the ratio of *erythro* and *threo* forms of β-O-4 structures during lignin formation.

Previously the authors reported that the *erythro*/*threo* ratio of β-O-4 structures in stepwise extracted milled wood lignins (MWLs) from a hardwood increased with the increase in milling time (Matsumoto et al., 1993). Although the effect of polymerization pattern of lignin precursors (Zutropf or Zulauf) was emphasized in their report, the result itself could sugest the role of aromatic ring type as the factor affecting *erythro*/*threo* ratio because it had already been known that the syringlyl/guaiacyl ratio (determined by nitrobenzene oxidation and elementary analysis) of MWLs prepared in the similar manner increased with the progress of milling time (Lee et al., 1981).

3. Experimental

3.1. Plant material

A 51-year-old Yellow Poplar tree (*Liriodendron tulipifera* L.) 14 m tall and 30 cm in diameter at breast height growing in West Virginia was used (Yoshida et al., 2002; Okuyama et al., 1994). This tree showed the highest longitudinal growth stress on the upper side of the leaning stem (Okuyama et al., 1994), indicating that this side is tension wood, which in this species does not have gelatinous fibers. Wood blocks consisting of 30–50 growth rings in sapwood were taken from 13 positions along the periphery of the wood disk as shown in Fig. 2, and were ground by a Wiley mill and extracted with EtOH–benzene (1:2) under reflux for 6 h by a Soxhlet apparatus. These extracted wood meals were subjected to the Klason procedure for the determination of lignin content, and methoxyl group contents of obtained Klason lignins were determined. The more fine wood meal was prepared from the extracted wood meal using a vibratory ball mill (Retsch type MM200) for 10–15 min with vibration of 30 s^{-1} and subjected to ozonation analysis and determination of methoxyl group contents in wood meal.

3.2. Chemicals

Cation exchange resin ($NH4^+$ form) was prepared from the H^+ form of the resin (Dowex-50W-X4, H^+ form). All other chemicals were of reagent grade.

3.3. Determination of lignin content

Lignin content were determined as the total amount of the insoluble part (Klason lignin) and the soluble part (acid soluble lignin) (Dence, 1992).

3.4. Determination of the ratio of erythro and threo forms of β-O-4 structures

The ratio of *erythro* and *threo* forms of β-O-4 structures in lignin was determined by ozonation analysis according to the method of Akiyama et al. (2002) except for sample weight (50 mg of wood meal), quantity of internal standard (10 μmol of erythritol) and reaction time of ozonation (2 h).

3.5. The determination of the methoxyl group content

The methoxyl group content was determined according to the method of Baker (1996). The following is the procedure modified by Goto et al. (2001). Hydroiodic acid (57% w/w, 10 ml) was sealed in a vial with Klason lignin (30 mg) or wood meal (60 mg). The vial was placed in a 130 °C oil bath for 20 min with shaking and then cooled in an ice bath. Ethyl iodide (0.1 mmol) in tetrachloromethane as an internal standard was added through the septum, followed by CCl_4 (15 ml) with cooling. The vial was shaken vigorously and an aliquot of CCl_4 phase was separated into a 2 ml vial with Na_2SO_4. The resulting methyl iodide yield was determined by GC analysis using a Shimadzu 14B gas chromatograph, equipped with a FID and a fused-silica capillary column (Varian CP-SIL 13CB for halocarbons 0.32 mm i.d. × 25 m). The oven temperature was held for 5 min at 40 °C, raised at 10 °C/min to 180 °C. The injector and detector heater temperature were 200 and 230 °C, respectively. The injection volume was 1.0 μl and the splitting ratio was 55:1. The flow-rate of He carrier gas was 1.1 ml/min.

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