論文の内容の要旨

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論文題目：Model Study on the Water Addition Reaction to Quinone Methide Intermediates during Lignin Biosynthesis

（リグニン生合成過程におけるキノンメチド中間体への水付加反応に関するモデル研究)

Chapter 1 Introduction

The β-O-4 structure is the most abundant structural linkage in lignin, which is formed through a β-O-4 radical coupling between monolignols or, and the phenolic end of a growing lignin, followed by water addition to the quinone methide (**QM**) intermediate. The resultant β-O-4 structures have two different isomeric forms, *erythro* (**E**) and *threo* (**T**) forms (**Fig. 1**).

Although the ratio of **E** and **T** forms is close to 50:50 in normal softwood lignin, the **E** form dominates in hardwood lignin. For **E** or **T** isomer to be produced in excess during lignin biosynthesis, stereo-preferential water-addition must occur to the planar **QM** intermediate (**Fig. 1**). What controls the diastereopreference during water-addition? Syringyl/guaiacyl (**S/G**) ratio of aromatic nuclei seems to be one of the factors governing this **E/T** ratio, which was indicated by NMR[1] and ozonation analysis[2], as well as by a model study on water addition to **S**-type-**QM** mimicking hardwood lignin biosynthesis.[3] There was a case, however, in which the **E/T**

Fig. 1 Formation of *erythro* and *threo* forms of β-O-4 structures through lignin end-wise biosynthesis.

ratio was not 50:50 in softwood lignin in spite of the absence of **S**-units. Previous studies showed that β-O-4 structures in the compression-wood lignin were slightly predominant in **E** form. [4, 5] The distribution of the **E/T** ratio in the reaction wood disc was positively correlated with that of *p*-hydroxyphenyl/guaiacyl (**H/G**) ratio, implying that the *erythro*-preferential formation is also related to the **H**-unit of aromatic ring type.[5] Nevertheless, direct model experimental evidence on this phenomenon has not been studied in detail.

In this thesis, model study mimicking compression-wood lignin biosynthesis was conducted to investigate factors determining the **E/T** ratio of **H**-**G** type lignin during rearomatization of **QM** intermediate. In detail, water addition to β-O-4 bonded **QM** models and synthesis of artificial lignin through end-wise coupling were independently performed by varying pH condition, solvent condition and chemical structures of either a **QM** moiety or a β-etherified aromatic ring in **QMs**, respectively.

Chapter 2 Synthesis of lignin β-O-4 models and related compounds

As shown in **Fig. 2**, several β-O-4 models were synthesized according to previous method.[3] The separation of *erythro* and *threo* isomers of each β-O-4 model was carried out using silica-gel chromatography or ion-exchange chromatography using a borate solution as the eluent. The configuration of each isomers of the β-O-4 models (*erythro* or *threo*) was determined by ozonation method, in which
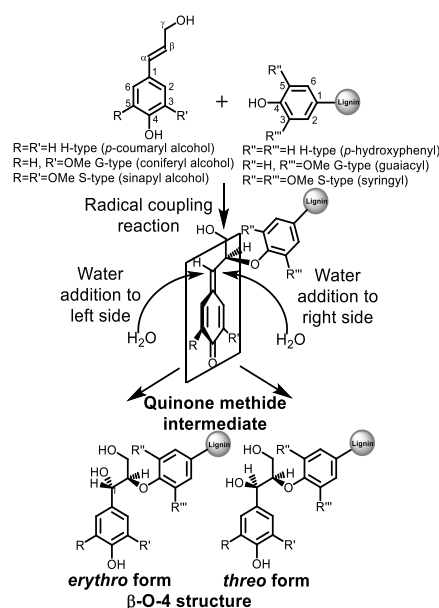
erythronic and threonic acid are obtained from the *erythro* and *threo* isomers, respectively. The β-O-4 models were used as the starting material to prepare **QM** models, and also used to quantify the β-O-4 products from water addition to **QMs** in chapter 3.
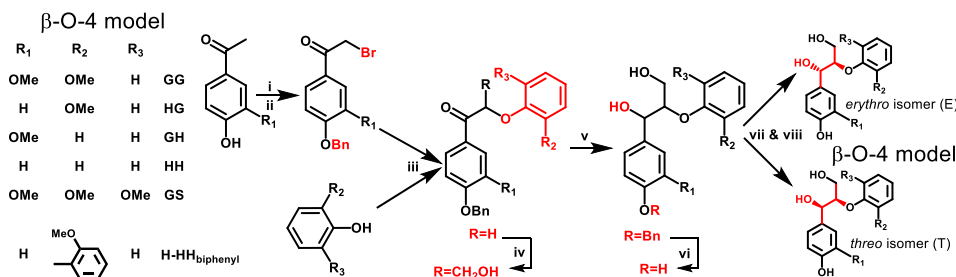

Fig. 2 Synthetic route of β-O-4 models. Reagents and conditions were as follows: (**i**) BnCl, KI, K_2CO_3, anhydrous DMF, 90 °C, (**ii**) Br_2, EtOH, rt, (**iii**) K_2CO_3, acetone, 40 °C, (**iv**) HCHO, THF, K_2CO_3, 40 °C, (**v**) $NaBH_4$, THF-EtOH (v/v, 1:2), rt (mixture of *erythro* and *threo* isomers), (**vi**) 10% Pd/C, H_2, THF, rt (mixture of *erythro* and *threo* isomers), (**vii**) separation of diastereomers, (**viii**) determination of separated β-O-4 model with ozonation method

Chapter 3 Water addition to β-O-4 bonded quinone methide models mimicking lignin biosynthesis
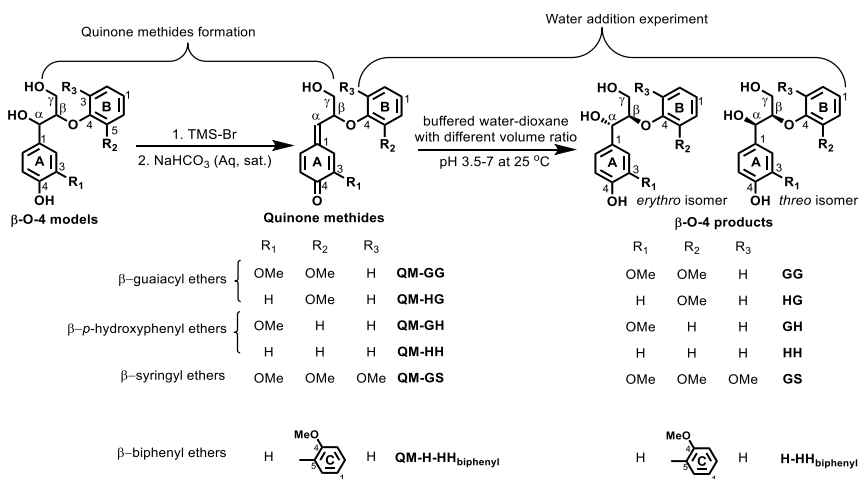

Fig.3 Preparation of **QM** compounds and water addition experiment to the **QMs** to form β-O-4 products obtained as a mixture of *erythro* and *threo* isomers. (**QM-GS** was used as a controlled experiment to compare with Brunow et al.'s results)

A series of **QMs** were synthesized from corresponding β-O-4 models and subjected to water in pH 3-7 or dioxane-water ratio 1/79-1/1, v/v at 25 °C (**Fig. 3**). Based on half-lives of QMs during water addition (**Table 1** and **2**), a *p*-hydroxyphenyl **QM** (**QM-HG** and **-HH**) can react with water faster than guaiacyl-type ones

Table 1. Half-lives for the disappearance of **QMs** in dioxane-water (1:1) under different pH conditions (s: second)

QM	pH value				
	3.5	4.5	5.5	6.0	7.0
QM-GG	84 s	460 s	791 s	850 s	876 s
QM-HG	<10 s	17 s	32 s	34 s	36 s
QM-GH	90 s	461 s	651 s	667 s	648 s
QM-HH	11 s	15 s	17 s	17 s	18 s

(**QM-GG** and **-GH**) in every condition employed here, which suggests that the rapid aromatization in **H**-type **QMs** possibly contributes to the development of highly lignified compression wood in nature. The reaction rates of water addition were largely dependent on the structural type of the **QM** moiety, whereas the stereo-selectivity was affected by the β-etherified aromatic ring structure greater than the

type of the **QM** moiety (**Fig. 4** and **5**). The β-O-4-aryl ether **QMs** bearing β-etherified guaiacyl ring yielded higher proportion of *erythro* isomer than ones carrying β-etherified *p*-hydroxyphenyl ring within the *threo*-selective aromatization for all β-O-4-aryl ether **QM** dimer. Furthermore, if the β-etherified ring of **H**-type **QMs** was changed with a **biphenyl** unit, *erythro*-preferential water addition can occur on an H-HH_{biphenyl} trimer (**Fig. 4**). This is same as the expectation based on the previous observation of the slight but clear *erythro* preference of β-O-4 structures in compression wood lignin.

Fig. 4 The proportion of *erythro* to *threo* isomers of β-O-4 products from water addition in different pH

For the effect of pH and solvent condition on the water addition to QMs, both lower pH condition and higher volume percentage of water can accelerate the reaction rate of water addition to any **QMs** in **Table 1** and **2**. We also could change the *erythro/threo* ratio of β-O-4 products by varying the pH or dioxane/water volume ratio during water addition reaction, but we still cannot get the *erythro*-dominated water addition products (**Fig. 4** and **5**). The proportion of *erythro* isomers was higher at lower pH condition (**Fig. 4**). Even though this effect of pH on the stereo-preferential formation of β-O-4 products from **QMs** can be observed in every solvent with different dioxane/water volume ratio, such effect is larger in the solvent containing 50% of dioxane (or 50% of water) than 1% of dioxane (or 99% of water) (**Fig. 5**). The *erythro/threo* ratio of β-O-4 products varied with different dioxane/water volume ratio

Table 2. Half-lives for the disappearance of **QMs** in different dioxane-water ratio under same pH conditions (s: second)

QM	pH	dioxane/ H$_2$O, (v/v)				
		1/79	1/7	1/5	1/1.5	1/1
QM-GG	5	81 s	146 s	157 s	448 s	839 s
	7	84 s	150 s	182 s	501 s	978 s
QM-HG	5	28 s	29 s	29 s	36 s	49 s
	7	26 s	28 s	29 s	41 s	59 s
QM-GH	5	77 s	126 s	130 s	398 s	759 s
	7	72 s	123 s	133 s	426 s	795 s
QM-HH	5	<20 s	<20 s	25 s	32 s	45 s
	7	<20 s	24 s	24 s	36 s	51 s
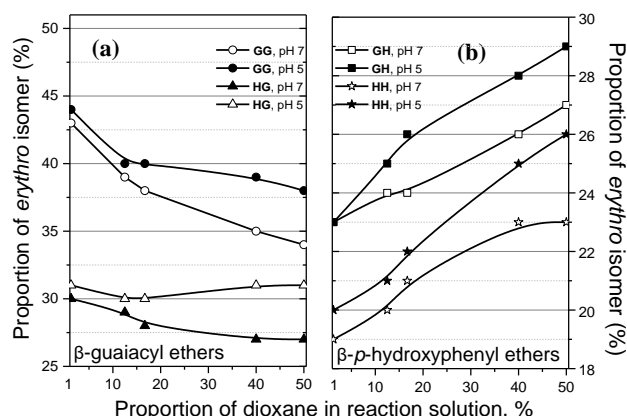
Fig. 5 The proportion of *erythro* to *threo* isomers of β-O-4 products from water addition in different dioxane-water ratio

and tended to opposite trend depending on the type of β-etherified aromatic ring in corresponding **QMs**. As shown in **Fig. 5a**, the *erythro/threo* ratio of β-O-4 products from **QMs** with β-etherified guaiacyl unit decreased as an increase of volume percentage of dioxane. In contrast, the *erythro/threo* ratio of β-O-4 products from **QMs** with β-etherified *p*-hydroxyphenyl ring showed an increasing trend (**Fig. 5b**). The pH- and dioxane/water ratio-dependent trend for water addition to **QMs** were suggested
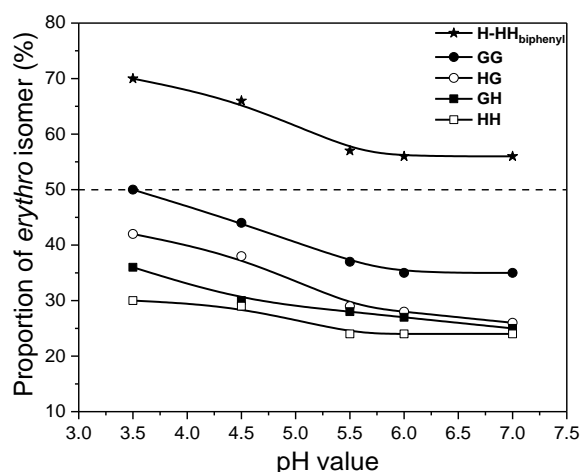
to be important factors for understanding and reproducing the condition in cell walls for lignification.

Chapter 4 Preparation and characterization of dehydrogenation polymers (DHP) from *p*-coumaryl alcohol

On the basis of results in Chapter 3, we propose the potential role of pH, solvent, β-etherified structures and its size on stereo-selectivity of water addition to β-O-4 ether quinone methide intermediate during lignin polymerization. Thus in this Chapter, a series of **H**-type **DHPs** were prepared from *p*-coumaryl alcohol in different reaction condition by using the end-wise or bulky polymerization method (**Table 3**). Based on the ozonation results in **Table 3**, both the pH-dependent and dioxane-water ratio-dependent trend for water addition experiment on QMs were not observed in these DHPs prepared in different pH or solvent condition. All of them have the approximate proportion of *erythro* form of 36-38%. While, in the respect of **QM** intermediates derived from β-O-4-coupling between a *p*-coumaryl alcohol and a phenolic end of the growing polymer (**Fig. 1**) during end-wise polymerization of **DHP**, the *erythro/threo* ratio of resultant β-O-4 structures were higher than the results from water addition experiments on **QM-HH** dimer (**Fig. 4** and **5**). Also, in **Table 3**, the **DHP 6** with high Molecular Weight (MW) prepared from end-wise polymerization contains more proportion of *erythro* form of β-O-4 structures than **DHP 7** with low MW prepared from bulky polymerization. Thus, it is appeared that there is a relationship between the size of β-etherified moiety of **QM** and stereo-preferential formation of its corresponding β-O-4 product. However, the *erythro*-preferential formation, which we observed on water addition experiment for **QM-H-HH$_{biphenyl}$** in **Fig. 4**, was not found in the β-O-4 structure of **DHPs** at any conditions employed here, the *threo* forms were still produced in excess during the polymerization of **DHPs**. It implies that the β-etherified aromatic ring of **QM** intermediate may have more chances to result the *erythro* form of β-O-4 structures with the *ortho*-substitutions than *para*-substitutions during water addition.

Table 3. The information of **H**-type **DHPs** prepared from *p*-coumaryl alcohol in different condition, Not Available[a]. MW is too large to dissolve in solvent for GPC analysis

DHP	pH	dioxane/water	polymerization	E+T (μmol/mg)	E/(E+T)	Mn	Mw	PDI
1	3.5	1:7	end-wise	0.288	37%	1642	4715	2.87
2	4.5	1:7	end-wise	0.313	37%	2022	5959	2.95
3	5.0	1:23	end-wise	0.323	36%	1593	9296	5.84
4	5.0	1:7	end-wise	0.346	36%	2780	11099	3.99
5	5.0	1:1.4	end-wise	0.309	38%	NA[a]	NA[a]	NA[a]
6	6.0	1:7	end-wise	0.253	36%	NA[a]	NA[a]	NA[a]
7	6.0	1:7	bulky	0.094	32%	1278	2250	1.76
8	7.0	1:7	end-wise	0.265	38%	NA[a]	NA[a]	NA[a]

Chapter 5 Summary

Such experiments in this paper may help us to further understanding of condition in cell walls for lignification. Notably, the pH, water-media solvent and *ortho*-position or size of β-etherified aromatic ring in **QM** intermediate play an important role on the lignin polymerization.

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