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Contribution of Syringyl β-O-4 Linkage in Different Hardwood Lignin Preparations

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Introduction

Chemical structure of lignin is known to possess different characteristics in different morphological regions and different lignification stages. Kinds of aromatic nuclei and inter-monomer linkages are most remarkable points for the study of heterogeneity of lignin.

For the study of lignin, some kinds of isolated lignins are generally used. Milled wood lignin (MWL) is commonly used for the structural study of lignin because it is considered not to suffer from chemical modification during preparation and it is readily soluble in organic solvents. However, the yield of MWL is not so high, and so it is not necessarily considered to represent whole lignin. As far as the origin of MWL, LEE *et al.* concluded that at least an initially extracted hardwood MWL originated from compound middle lamella.[6] On the other hand, TERASHIMA *et al.* reported that milled wood lignin originated from the secondary wall rather than from the middle lamella by radiotracer experiment.[9]

Periodate lignin is an isolated lignin whose yield is sufficiently high and chemical modification is relatively low. This is the reason why periodate lignin has been used as lignin sample in the series of this study.

β-O-4 is the most major linkage between phenylpropane structural units in lignin and in a β-O-4 rich fraction found by FUKAGAWA *et al.* in birch periodate lignin, was reported to be 80% of linkages were composed of β-O-4 linkage.[2] This fraction was a part of 90% aqueous dioxane soluble fraction of the periodate lignin. In the 90% aqueous dioxane insoluble part of birch periodate lignin, a similar β-O-4 rich lignin fraction was obtained by the partial cleavage of β-O-4 linkage with TMSiI.[4] In the previous paper, the endwise lignin fraction separated from birch periodate lignin was treated with TMSiI and sinapyl alcohol was found in the reaction mixture as a main product.[5] Sinapyl alcohol is a expected compound produced by the cleavage of β-O-4 linkage with TMSiI.

In this paper, TMSiI treatment was applied to various lignin these lignins.

Experimental

Lignin preparations from birch wood

Birch periodate lignin (PIL) and milled wood lignin (MWL) were prepared according to the previous paper.[4] Periodate lignin was extracted with 90% aqueous dioxane to obtain solvent soluble fraction. After acetylation, the solvent soluble fraction was extracted with methanol and then endwise lignin fraction was obtained as the methanol insoluble fraction.

Preparation of wood fiber lignin

Birch wood chips were pre-steamed at 110℃ for 10 min and defibrated by a pressurized refiner and mechanically defibrated wood was obtained. The defibrated wood was

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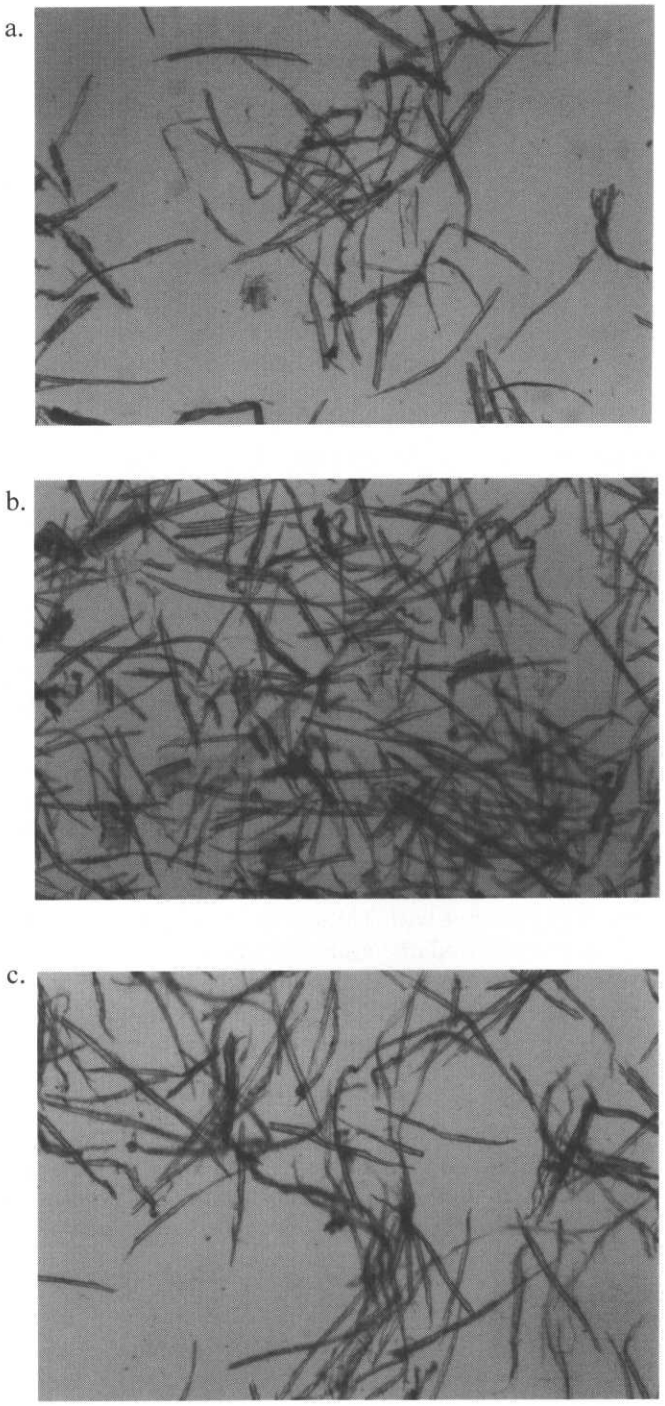

Photo 1. The photos of the wood fiber fraction and the other fraction. a. 40–100 mesh fraction; b. 40 mesh on fraction; c. 40–50 mesh fraction (wood fiber fraction).

Table 1. Characterization of lignin in the wood fiber fraction by alkaline nitrobenzene oxidation.

	Wood chip	Wood fiber fraction
Lignin content (%)	23.1	23.2
Total aldehyde yield (%)	55.4	61.5
S/V ratio	3.17	3.68

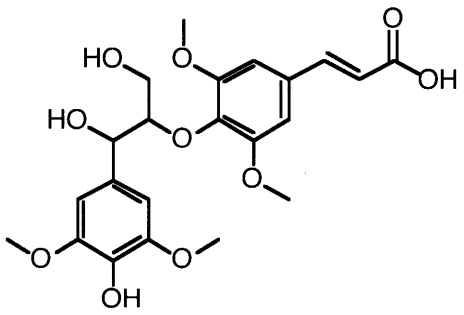

Fig. 1. Chemical structure of syringyl β-O-4 type dimeric model compound.

then fractionated by sieving with different meshes. A fraction of 40 mesh pass and 100 mesh on was collected to obtain wood fiber fraction (Photo 1a). However, degraded vessel fragments still remained considerably as small fragments in this fraction, although vessels and bundles were almost removed compared with the case of 40 mesh on (Photo 1b). Then a fraction of 40 mesh pass and 50 mesh on was collected. It was confirmed that this fraction was almost wood fiber fraction as shown in Photo. 1c. The characteristics of the wood fiber fraction are higher total aldehyde yield and higher S/V molar ratio, indicating the nature of less condensed and relatively rich in syringyl unit (Table 1). Periodate lignins were also prepared from the mechanically defibrated wood and the wood fiber fraction, and named as periodate lignin of defibrated wood and periodate lignin of wood fiber.

Synthesis of syringyl type lignin model compound

A syringyl β-O-4 type dimeric lignin model compound, 4-[2-(3,5-dimethoxyphenyl-4-hydroxy)-2-hydroxy-1-(hydroxymethyl)ethyl]sinapinc acid (Fig. 1), was synthesized according to the method of β-O-4 dimeric model synthesis by HELM *et al.*[3] Acetosyringone and sinapic acid were used as the starting material in this study.

TMSiI treatment of lignin fractions

Trimethylsilyl iodide (TMSiI) was purchased from Aldrich Chemical Co., U.S.A. and was stored in a refrigerator.

One milliliter of chloroform containing 10 mg acetylated lignin (or lignin model compound) was sealed in a glass-tube with a silicone rubber cap and cooled in a cold bath maintained at 0℃ for 30 min. A 40 μl of TMSiI was added quickly to the above solution by the use of a syringe through the silicone rubber cap and the mixture was kept in the same cold bath with gentle stirring for 3 hours unless otherwise mentioned. The reaction was terminated by the addition of small amount of pyridine and then water containing $Na_2S_2O_3$. The mixture was kept in the cold bath for 30 minutes to stop completely the reaction. Then, in the case of quantitative determination of sinapyl alcohol, acetoguaiacone in chloroform solution was added as the internal standard for GC analysis. After acidification by 2 M HCl, the reaction products were extracted with chloroform exhaustively and analyzed by the use of GC, and GC-MS.

GC, GC-MS, and GPC conditions: same to those described in the previous paper.[5] Sinapyl alcohol was determined by GC with acetoguaiacone as an internal standard.

Results and Discussion

Cleavage of syringyl β-O-4 model compound with TMSiI treatment

In the previous paper, an endwise lignin fraction obtained from birch periodate lignin

was treated with TMSiI and sinapyl alcohol was confirmed in the reaction mixture as a main product.[5] Based on the reaction mechanism for the cleavage of β-O-4 ether linkage by TMSiI proposed in the earlier paper,[7] it was believed to be originated from lignin substructures linked by two sequential β-O-4 ether linkages.

However, question was still remained. Coniferyl alcohol which was expected from guaiacylglycerol-β-guaiacyl ether (GG) by TMSiI treatment was not always confirmed in the reaction products, although several products attributable to the further reaction of coniferyl alcohol were detected as reported in the previous paper.[5] Therefore, in this paper, a syringyl-syringyl type β-ether model compound was synthesized and applied to TMSiI treatment. Syringylglycerol-β-sinapic acid ether was treated with TMSiI under the condition of 0℃, 3 hr. Sinapyl alcohol was confirmed in the reaction products by GC and GC-MS. Yield of sinapyl alcohol was 39.6 mol%. It is considered that sinapyl alcohol is a major product of the TMSiI treatment for syringyl β-O-4 model compound.

TMSiI treatment products from endwise lignin fraction

GPC chromatogram of endwise lignin fraction after different periods of TMSiI treatment are shown in Fig. 2. A sharp peak appearing at the elution volume of 25.4 m*l* was confirmed to be sinapyl alcohol by GC-MS analysis,[5] which became smaller with the TMSiI treatment time. It is obvious that sinapyl alcohol formed by the cleavage of β-O-4 ether structure in lignin is not stable under the treatment condition and is involved in the further reactions. The peak around 17 m*l* may indicate that the reaction has not completed yet at 3 hr. After 6 hr, the peak disappeared, and another broad peak at around 22 m*l* started to increase (data are not shown). When the treatment time was extended to 24 hr, the broad peak became quite remarkable, indicating considerable condensation of reaction products.

GC chromatogram of TMSiI treatment products of endwise lignin fraction showed the formation of sinapyl alcohol and very small amount of sinapaldehyde. As shown in Fig. 3, yields of these two reaction products decreased rapidly with reaction time.

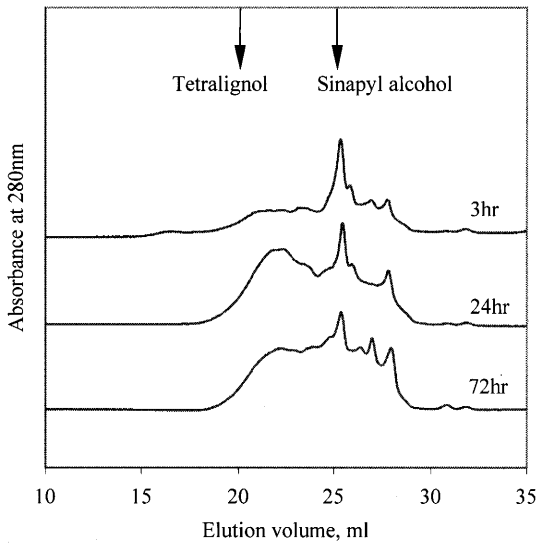

Fig. 2. GPC curves of endwise lignin fraction treated with TMSiI for different period.

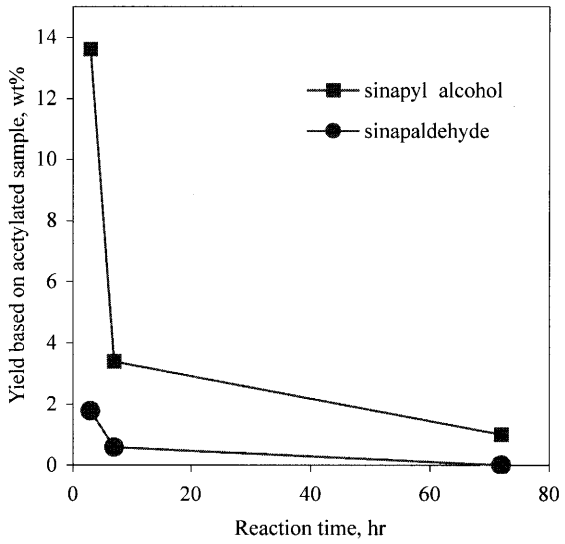

Fig. 3. Yield of sinapyl alcohol and sinapaldehyde from endwise lignin fraction by TMSiI treatment. (Data points are 3, 7 and 72 hr, respectively)

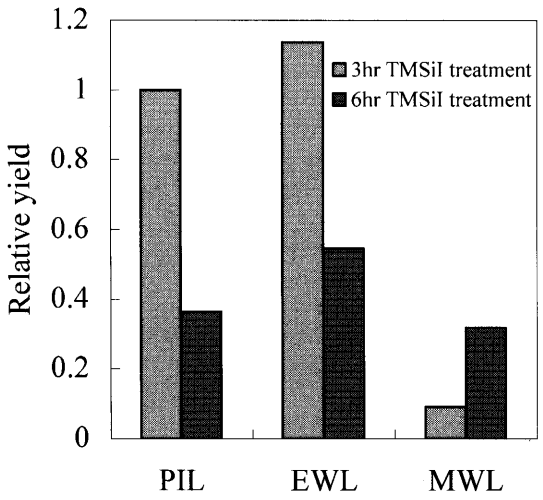

Fig. 4. Comparison of relative yield of sinapyl alcohol between various lignin samples. PIL: periodate lignin, EWL: endwise lignin fraction, MWL: milled wood lignin.

TMSiI treatment of different lignin preparations

Three different lignin preparations from birch wood meals, namely periodate lignin, endwise lignin fraction of periodate lignin, and MWL, were treated with TMSiI under the same reaction conditions, and the relative yields of sinapyl alcohol by 3 and 6 hour reaction were shown in Fig. 4. It is obvious that yield of sinapyl alcohol from endwise lignin fraction was apparently higher than that from periodate lignin, indicating the characteristic nature of endwise lignin fraction. It is interesting to note that yield of sinapyl alcohol from MWL was remarkably lower than whole periodate lignin and endwise lignin fraction. This fact means that MWL does not have an average nature of whole lignin but a special part of

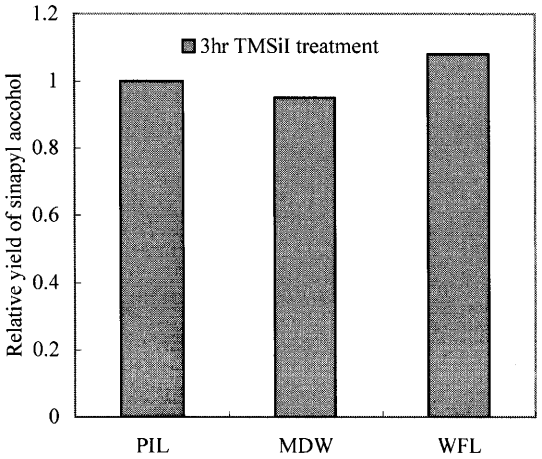

Fig. 5. Comparison of rerative yield of sinapyl alcohol between whole lignin and wood fiber lignin. PIL: periodate lignin of wood chip, MDW: periodate lignin of mechanically defibrated wood, WFL: periodate lignin of wood fiber separated from mechanically defibrated wood.

lignin. In the case of MWL, a higher yield of sinapyl alcohol was found at 6 hr than at 3 hr, although it is difficult to explain at the moment. In order to discuss the structural differences among lignin due to morphological difference, three different periodate lignin from wood meal, defibrated wood and wood fiber fraction were treated separately with TMSiI, and the relative yields of sinapyl alcohol by 3 hour reaction are shown in Fig. 5. Periodate lignin from wood fiber fraction gave higher yield of sinapyl alcohol than the other two lignins. This result is consistent with the result of alkaline nitrobenzene oxidation (Table 1). This may indicate syringyl β-O-4 structure rich nature of wood fiber lignin. Fergus and Goring confirmed that wood fiber lignin was syringyl rich and vessel lignin was guaiacyl rich by UV microscopy.[1] SAKA *et al.* reported that the syringyl-guaiacyl ratio of wood fiber lignin was 88 : 12 and the ratio of vessel lignin was 12 : 88 by the bromine EM-EDXA method.[8] The results obtained in this study by TMSiI treatment are in good accordance with the earlier papers.

Conclusion

1. Sinapyl alcohol is a major product of the TMSiI treatment for syringyl β-O-4 model compound.
2. Two isolated lignins, periodate lignin and milled wood lignin obtained from the same wood, exhibit different natures in terms of the yields of sinapyl alcohol. This may indicate that milled wood lignin is a special part of whole lignin.
3. Wood fiber lignin behaves as syringyl β-O-4 rich lignin by TMSiI treatment. Both nitrobenzene oxidation of wood fiber fraction and TMSiI treatment of periodate lignin obtained from the same fraction suggested that wood fiber lignin is syringyl β-O-4 rich lignin.

Summary

Sinapyl alcohol was confirmed as a major product of trimethylsilyl iodide (TMSiI) treatment of syringyl β-O-4 lignin model compound. In this study, contribution of syringyl β-O-4 linkage in different lignin preparations was investigated by comparing the yields of

sinapyl alcohol obtained by TMSiI treatment. Milled wood lignin (MWL) produced lower yield of sinapyl alcohol than periodate lignin of birch wood chip, suggesting that MWL was a special part of whole lignin. Periodate lignin of wood fiber was confirmed to be rich in syringyl β-O-4 because it gave higher yield of sinapyl alcohol than periodate lignin obtain from whole wood.

Key words: Endwise lignin, Trimethylsilyl iodide, β-ether cleavage, α-condensation, iodination

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異なる広葉樹リグニンでのシリンギルβ-O-4結合の寄与

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要　旨

シリンギル，β-O-4型リグニンモデル化合物のトリメチルシリルアイオダイド(TMSiI)処理において，シナピルアルコールが主要生成物であることが確認された。TMSiI処理によるシナピルアルコールの収率を比較することにより，さまざまなリグニン中のシリンギルβ-O-4結合の寄与について検討した。一般的な単離リグニンである磨砕リグニン(MWL)は，過ヨウ素酸リグニンに比べシナピルアルコールの収率が低く，特別なリグニンであるということが示唆された。また，木繊維の過ヨウ素酸リグニンは木材全体の過ヨウ素酸リグニンに比べシナピルアルコールの収率が高く，よりシリンギルβ-O-4の多いリグニンであるということが示唆される結果となった。

キーワード：エンドワイズリグニン，トリメチルシリルアイオダイド，β-エーテル開裂，α縮合，ヨウ素化

Study on the Relationship between Experiences in Nature and Recognition of Animals and Plants in Childhood

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The most important purpose of this paper is the clarification of the relationship between experiences of nature and recognition of plants and animals in childhood. First, the change of environment in the study area was surveyed. Next, the play and lifestyle (especially using wildlife and plants) of the examinees' childhood was examined using on-the-spot fact-finding. Finally, the relationship between childhood experiences and recognition of wildlife was considered for a number of categories of plants and animals. Each category was found to have its own characteristic recognition factor. For example, recognition of fish, insects and plants were strongly connected with play, and in many cases insects and plants were found to symbolize specific seasons.

Contribution of Syringyl β-O-4 Linkage in Different Hardwood Lignin Preparations

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Sinapyl alcohol was confirmed as being a major product of trimethylsilyl iodide (TMSiI) treatment of the syringyl β-O-4 lignin model compound. In this study, the contribution of syringyl β-O-4 linkage in different lignin preparations was investigated by comparing the yields of sinapyl alcohol obtained by TMSiI treatment. Milled wood lignin (MWL) produced a lower yield of sinapyl alcohol than periodate lignin from birch wood chips, suggesting that MWL was a special part of the whole lignin. Periodate lignin of wood fiber was confirmed to be rich in syringyl β-O-4 because it gave higher yields of sinapyl alcohol than periodate lignin obtained from whole wood.