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<td>研究は新規のβ-O-4切断方法の選択的β-オキシラン分解反応を助ける硫黄群-γ-TTSA法による。</td>
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Studies on the novel selective β-O-4 cleavage method of lignins by E1cB type elimination reaction assisted by the sulfone group -γ-TTSA method-

- Abstract version -

2014
Daisuke Ando
Introduction

Petroleum-based chemical products are indispensable for the chemical industry today. However, the fossil refinery processes give rise to some environmental problems, such as degradability and toxicity. In addition, the price of petroleum has risen steadily for several years. Thus, our society is facing the shift from a fossil-fuel-based society to a new sustainable society that is based on renewable resources, such as biomass. Especially, wood biomass, representing about 146 billion tons a year, is drawing attention. The sustainable use of bio-based resources suggests integrated manufacturing in biorefineries. Wood biomass is a polymer complex consisting of biomaclomolcules, such as cellulose, lignin and hemicellulose. Thus, the utilization of each component according to its feature is needed. The biorefinery concept embraces a wide range of technologies able to separate biomass into the building blocks, which can be converted to value-added products, chemicals, and biofuels (Cherubini and Stromman 2011). However, the development and application of lignocellulosic biomass fractionation technologies that are technically and economically feasible is still in its infancy. That is, the biorefinery of wood biomass should be ideally initiated with the highly selective separation of their components, i.e., cellulose, hemicellulose and lignins, without large chemical modifications which have been carried out by the conventional chemical modification of wood, such as wood-pulping and saccharification.

Of these components in wood biomass, lignin is the key component for biorefinery because lignin is connected between microfibrils in a plant cell wall. Lignin is, after cellulose, the second abundant biomacromolecule as natural organic materials and comprised about 20-30% of woods and 15-20% of grasses. Its structure is built of phenylpropanoid units, linked with various types of ether and carbon-carbon bonds and gives stiffness, strength, waterproof and rotproof of the cell wall (Boerjan et al. 2003). However, the lignin’s chemical structure has not yet been completely elucidated because some different kinds of bonds are formed by random-coupling of some radicals derived from monolignols. That is, lignin’s chemical structure and features are quite different from polysaccharides.
Thus, it is considered that a separation of biomass to lignin and polysaccharide is the first step for biorefinery. Therefore, the knowledge of structures between components is needed in these days. Indeed, there are the interactions between the components, such as entangling and chemical bonding. Especially, the chemical bondings between lignin and polysaccharide, called Lignin-Carbohydrate-Complex (LCC), have been studied for many years in lignin chemistry. Several chemical bonds have been proposed to be prevalent between lignin and polysaccharides such as phenyl glycoside, benzyl ester and benzyl ether linkages (Watanabe 1995; Koshijima and Watanabe 2003; Lawoko et al. 2005; Balakshin et al. 2007, 2011). Many investigations have been conducted to isolate and elucidate the LCC’s chemical structure. Besides the above industrial interest, studies on the chemical structure of LCC in wood are of significance for the elucidation of the LCC biosynthesis in cell-wall construction. Much knowledge is obtained by degradation techniques such as alkaline hydrolysis, acid hydrolysis and Smith degradation. In these approaches, wood samples are degraded to afford the resulting products, followed by identification of the products. The useful information could be got in spite of indirect evidence. Moreover, valuable information of LCC’s type and quantity in biomass has been obtained without any modification by using 2D-NMR analysis recently. However LCC’s structures are still unknown despite many researches. In past methods, information was lost by cleavage of LCC linkages in degradation approaches and knowledge about low-content LCC linkages is undetectable in 2D-NMR analysis. Therefore, the isolation and chemical structural determination of LCC bonding sites is an important approach for new knowledge. Indeed, direct proofs such as the isolation of LCC bonding site is not yet reported.

The selective degradation of each component is needed for LCC isolation. So, there are two approaches. One is an approach to degrade in order of polysaccharide, lignin. The other is an approach to decompose in order of lignin, polysaccharide. It is considered that the latter is useful for this purpose. This is because the obtained degradation products of the latter have easier structure than that of the
The key point is to degrade lignin, that has the complicated structure, in the first step. Thereby, the polysaccharide-rich degradation products can be obtained in high molecular weight moiety. In subsequent step, the degradation products, such as LCC bonding sites and oligosaccharide, can be separated easily. However, there are no lignin degradation methods, in course of which LCC linkages are intact without damaging. A novel selective degradation method for LCC analysis is needed.

In order to clarify the exact chemical structure of lignin on the basis of intact degradation products of it and to obtain the LCC molecules, such as that linked with benzyl ether, the author has developed the novel selective degradation method for β-O-4 substructures in lignin, named as γ-TTSA method which consists of four steps; selective tosylation (T) of a primary hydroxyl group at γ-position, subsequent thioetherification (T), sulfonylation (S) and mild alkali treatment (A).

Thus, in this study, two lignin degradation methods (α-TSA method and γ-TTSA method) were established in model experiment. In α-TSA methods, it was clear that β-elimination reaction of sulfone compound is appropriate for the β-O-4 cleavage. Then, the utilities and detail reaction mechanism of the γ-TTSA method for LCC chemical structure analysis were investigated with using lignin model compounds; dimeric β-O-4 lignin model compounds, artificial lignin (dehydrogenative polymer: DHP). And then, purified milled wood lignin (pMWL) as native lignin was also analyzed by γ-TTSA method. Finally, LCC in crude MWL (cMWL) was analyzed by γ-TTSA method. These results were summarized in five chapters.

References
Abstract

In the thesis, the author has demonstrated the development and the usefulness of γ-TTSA method for the structural analysis of LCCs. α-TSA reaction sequence, named as α-TSA method, was established. It demonstrated that the cleavage of β-O-4 linkage proceeded under mild alkali treatment without side reactions by the existence of a sulfone group at a neighboring carbon of the β-O-4 linkage (Chapter 1). Based on this result, γ-TTSA reaction sequence, named as γ-TTSA method for LCC analysis, was established in dimer model experiment. In γ-TTSA method, the structures at α-position in lignins, such as benzyl ether-type LCC bonding site, are retained (Chapter 2). In polymer model experiment, it was demonstrated that each reaction proceeded without any difficulty and β-O-4 linkages were cleaved selectively (Chapter 3). Indeed, when γ-TTSA method was applied to purified MWL, β-O-4 linkages were cleaved without damaging the other structure and its molecular weight decreased (Chapter 4). Accordingly, it was demonstrated that γ-TTSA method was useful for lignin degradation. Finally, lignin in crude MWL containing xylan was degraded by γ-TTSA method to obtain some LCC fractions from the degradation products. The new knowledge of LCC was obtained by the chemical structural analysis of the fractions (Chapter 5). From these results, it was insisted that γ-TTSA method was a useful lignin degradation method for LCC analysis. In the future, the information obtained by γ-TTSA method will contribute to investigate the chemical structures about LCC bonding sites. The following is summarized results. The outline of this thesis is shown in Figure 1.
Chapter 1. Cleavage of the β-O-4 linkage by the effect of sulfone group
The cleavage of β-O-4 linkage proceeded without side reactions by β-elimination of α-sulfone derivative. It found that the cleavage of β-O-4 linkage associated by sulfone group at neighboring carbon of the linkage.

Chapter 2. Novel lignin degradation method for LCC analysis -dimer model-
γ-TTSA method were established. The introduction of sulfone group to γ-position and subsequent cleavage of β-O-4 linkage were succeeded in lignin model dimer. The method was useful for LCC analysis because side reactions at α-position were not proceeded.

Chapter 3. Novel lignin degradation method for LCC analysis -polymer model-
In polymer model experiment with artificial lignin (DHP), β-O-4 linkage was cleaved selectively and other linkages were intact under γ-TTSA reaction sequence. As a result, molecular weight of DHP was decreased. Accordingly, it found that γ-TTSA method was useful for lignin polymer.

Application of γ-TTSA method

Chapter 4. Degradation of Native lignin
The purified MWL (pMWL) from *Eucalyptus globulus* was degraded by γ-TTSA method. β-O-4 linkages in pMWL were cleaved selectively and molecular weight of pMWL was decreased. As expected, lignin was degraded by the cleavage of β-O-4 linkages.

Chapter 5. Degradation of LCC
LCC (crude MWL) from *Eucalyptus globulus* was degraded by γ-TTSA method to obtain the degradation products. Then, their structures were analyzed in detail.

Figure 1 Schematic representation of the outline of this thesis
In Chapter 1, the author described the development of a novel lignin degradation method named as α-TSA method. This method consists of three steps; α-thioetherification (T) at the α-position, sulfonation by oxidation (S) and alkali treatment (A). β-O-4 linkage was quantitatively cleaved by α-TSA method in the application to guaiacyl type lignin dimer model compound to obtain E- and Z- α-β unsaturated sulfone compounds. The β-O-4 cleavage reaction proceeded with a mild alkali treatment without side reactions unlike conventional methods (acidolysis, thioacidolysis and DFRC etc.) with strong acid and high temperature conditions. Thus, it was demonstrated that β-elimination of sulfone compound was able to cleave the β-O-4 linkage under the mild condition without side reactions and then the α-TSA method was useful for the chemical structural analysis of lignin. These detailed results will be published in Holzforschung in 2014, (accepted).

In Chapter 2, the author described the development of a novel lignin degradation method for the analysis of LCCs, named as γ-TTSA method. The method consists of four steps; tosylation (T) of a primary hydroxyl group at γ-position, subsequent thioetherification (T) (substitution reaction with Dod-SH), sulfonylation (S) by oxidation with oxone and the cleavage of β-O-4 linkage by mild alkali treatment (A). In three non-phenolic β-O-4 lignin dimer model compounds guaiacyl, syringyl and p-hydroxylphenyl type, the β-O-4 linkages were quantitatively cleaved by γ-TTSA method to afford γ-β olefinic sulfone compounds. The β-O-4 cleavage initiated from the γ-position retains some information about the α-linkage. Thus, it was demonstrated that γ-TTSA method was useful for isolation of LCC bonding sites, where the α-position of lignin is involved in linkages to polysaccharides. These detailed results are published in Holzforschung (2012) 66: 331-339.

In Chapter 3, the author described the influence of other substructures except β-O-4 substructure and the reactivity of polymer in TTSA reaction sequence using artificial lignin, a so-called
dehydrogenation polymer (DHP). The obtained DHP according to the Zutropf method consists of β-O-4, β-5 and β-β substructures. Each reaction step of the method was followed by FT-IR and HSQC-NMR analyses. Tosylation proceeded selectively at the primary hydroxyl groups at γ-position of β-O-4 and β-5 substructures. Subsequent thioetherification and sulfonylation proceeded quantitatively. The β-O-4 and β-5 substructures in DHP were transformed to γ-sulfone derivatives without any side chain. Then, β-O-4 linkages were cleaved selectively by mild alkali treatment. On the other hand, β-5 and β-β linkages were intact after γ-TTSA treatment. In GPC analysis, it was found that molecular weight was decreased after γ-TTSA method. It is reconfirmed that γ-TTSA method is useful for the degradation of lignin polymer. These detailed results are published in Holzforschung (2013) 67: 249-256.

In Chapter 4, the author described the degradation of purified MWL (pMWL1) from *Eucalyptus globulus* by γ-TTSA method. The MWL consist of β-O-4 and β-β substructures mainly. β-O-4 substructures in pMWL1 were transformed to γ-sulfone derivatives without any side chain. In final step, the β-O-4 linkages were cleaved selectively without side reactions unlike the conventional methods. The reaction sequence was followed by HSQC-NMR analysis. Moreover, the peak at 570 Da of the expected degradation product and the peak at 1260 Da of degradation oligomer were found in GPC chromatogram of the obtained degradation product (pMWL5). The Mw of pMWL1 is decreased indicating the break down of the macromolecule to oligomers or monomers. As a consequence, natural lignin can be degraded by γ-TTSA method without the transformation of the structure at α-position. These detailed results are published in Holzforschung (2013) 67: 835-842.

In Chapter 5, the author described that lignin in crude MWL (cMWL1) from *Eucalyptus globulus* was selectively degraded by γ-TTSA method. The cMWL1 contains β-O-4 and β-β
substructures in lignin moiety and xylan in polysaccharide. Tosylation proceeded selectively at γ-position of β-O-4 substructure. For this, β-O-4 substructures in cMWL1 were transformed to γ-sulfone derivatives without the transformation of the other structures and the following alkali treatment cleaved almost the β-O-4 linkages selectively. Then, the new knowledge of LCC was obtained by the chemical structural analysis of the obtained degradation products. These detailed results will be submitted to Holzforschung.
**Publications**

The present study has been reported in the following papers.


