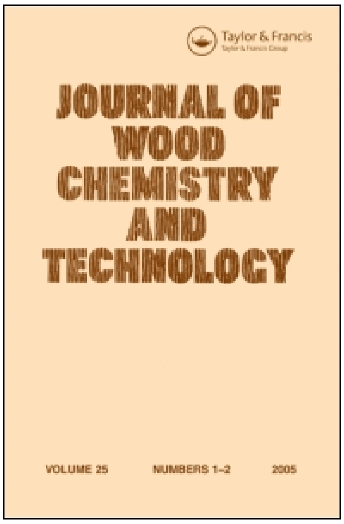

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Abstract: Dimeric lignin model compounds with non-phenolic and phenolic moieties and a β-O-4 type artificial lignin polymer were subjected to thioacidolysis to evaluate the reaction efficiency of thioacidolysis for cleavage of β-O-4 interunitary linkage. The obtained yields of thioethylated monomeric products from the dimeric lignin model compounds reached nearly 100% under the conventional condition, whereas that from the artificial lignin polymer was as low as 74%. Neither prolonged reaction time nor increased concentration of ethanethiol (the thioacidolysis reagent) enhanced the resulting monomer yields from the polymer (69–79%). Thioacidolysis of the lignin model compounds followed by HPSEC analysis also showed the dimeric model compounds were degraded almost quantitatively, but that the artificial lignin polymer was not. Thioacidolysis followed by desulfurization gave at least one dimeric product resulting from incomplete β-O-4 cleavage at significant yield. These results suggested the conventional thioacidolysis could not achieve quantitative cleavage of β-O-4 linkages in lignin macromolecules.

Keywords: Artificial lignin polymer, β-O-4 linkage, cleavage of ethereal bond, size exclusion chromatography (SEC), thioacidolysis

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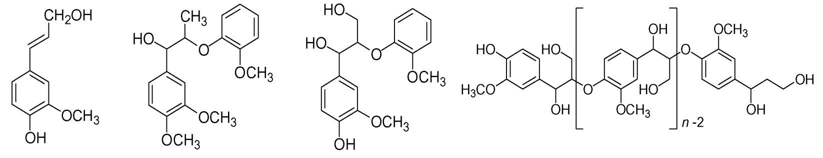
INTRODUCTION

Some wet chemical degradation methods, such as nitrobenzene oxidation,[1] permanganate oxidation,[2] hydrogenolysis,[3] acidolysis,[4] thioacidolysis,[5] ozonation,[6,7] DFRC,[8,9] and TIZ method,[10] have been proposed and employed for understanding lignin structures. Interunitary linkages in a lignin preparation are cleaved by those methods to provide some information on the frequency of the linkages from its low-molecular-weight degradation products. Of those chemical degradation methods, thioacidolysis has often received much attention.[11–13] The frequency of β-O-4 linkages was calculated from the types and the yields of monomeric products obtained by thioacidolysis.[14] Thioacidolysis also gave the information on the ratio of syringyl to guaiacyl units (S/G ratio) and on the frequency of interunitary linkages other than β-O-4 linkage concerning to condensed structures. The latter was obtained from dimeric products by thioacidolysis followed by desulfurization.[15] In addition, the number of β-O-4 bonds having a free-phenolic moiety at a terminal position of a lignin preparation was obtained by a modified thioacidolysis approach.[16] These information was based on an assumption that thioacidolysis cleaves aryl-ether bonds, β-O-4 interunitary bonds in particular, selectively and quantitatively.

However, it is apparently challenging to cleave β-ether bonds completely by thioacidolysis.[17] Although Lapierre stated in personal communication that β-ether dimer of incomplete ether cleavage rarely result when conditions were carefully optimized, Ralph and Grabber pointed out that thioacidolysis products of incomplete ether cleavage could occur to some extent.[17] Actually no complete cleavage of a β-*O*-4 dimeric model compound was shown in the literature.[17] Accordingly, it is necessary to clarify the relationship between reaction efficiency for cleavage of β-*O*-4 bonds and the reaction conditions of thioacidolysis, using a lignin model compound that surely mimics the β-*O*-4 interunitary linkages and the polymeric nature of lignin at the same time.

Recently, we have successfully developed a facile method to synthesize three kinds (i.e., *p*-hydroxyphenyl, guaiacyl, and syringyl moieties within the molecule) of artificial lignin model polymer, comprised exclusively of β-O-4 interunitary linkages.[18] The authors believe these polymers are more suitable to investigate reaction efficiency of analytical methods in lignin chemistry than dimeric model compounds of lignin that have been used conventionally.

In this study, the reaction efficiency of thioacidolysis for cleavage of β-*O*-4 bonds was estimated by using a β-*O*-4 type artificial lignin model polymer having guaiacyl nuclei only as well as non-phenolic and phenolic lignin model dimers. Next, the relationship between the reaction efficiency of thioacidolysis and its reaction conditions was also discussed.



CA (MW=180)

Compound Ia (MW=318)

Compound Ib (MW=320)

Compound II

Figure 1. Chemical structure of lignin model compounds used in this study. MW indicates molecular weight.

EXPERIMENTAL

Synthesis of Lignin Model Compounds

Veratrylglycerol-β-guaiacyl type ether, as a non-phenolic, dimeric lignin model compound, was prepared by the modified method[19] of Adler et al.,[20] then methylol group was converted to methyl group (as Compound Ia in Figure 1) by tosylation and iodations at γ-position of the side chain,[21] followed by Pd-C reduction in 90% aqueous 1,4-dioxane containing sodium bicarbonate as a catalyst. Guaiacylglycerol-β-guaiacyl ethers (Compound Ib), as a phenolic, dimeric lignin model compound was prepared according to the earlier work.[22] A β-*O*-4 type artificial lignin polymer consisting only of guaiacyl nuclei (Compound II) was prepared from acetoguaiacone.[18] Coniferyl alcohol (CA) was also prepared[23] and used as a monomeric lignin model compound.

Thioacidolysis Under the Conventional Workup Condition[13]

A lignin model compound (3–5 mg) with *n*-docosane (0.5 mg) as the internal standard was placed in a test tube under nitrogen atmosphere, and then 5.0 mL of 1,4-dioxane/ethanethiol (9:1, v/v) solution containing 0.4 M ethanethiol with boron tribromide etherate (0.1 mL) was added. The test tube was sealed and the thioacidolysis reaction was initiated by heating the mixture at 100°C in an oil bath, kept at the temperature for 4–24 h, and cooled on an ice bath. The reaction was quenched by the addition of 0.4 M sodium bicarbonate solution (2.5 mL). The resulting mixture was neutralized with 1.0 M hydrochloric acid to adjust the pH to 3.0. The mixture was extracted with dichloromethane (3 × 5 mL) and the organic layer was dried over sodium sulfate. The dichloromethane extract was concentrated under reduced pressure to a volume of 1.0 mL to serve the following gas chromatographic (GC) and high performance size exclusion chromatographic (HPSEC) analyses.

GC Analyses of Thioacidolysis Products

To an aliquot (30 μL) of the concentrated dichloromethane extract, pyridine (30 μL) and then N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA; 30 μL) was added. The extract was derivatized in a sealed vial at room temperature for 30 min. The resulting trimethylsililated [TMS] derivatives were subjected to the following gas chromatographic analyses, using a flame ionization detector (GC/FID) and a mass spectrometer (GC/MS).

The gas chromatographic analyses (GC/FID and GC/MS) were carried out on Shimadzu GC-2010 (Shimadzu Co. Ltd., Kyoto, Japan) and Shimadzu GCMS-QP2010 (Shimadzu Co., Ltd., Kyoto, Japan), respectively, under the same analytical condition. A non-polar, fused silica-based capillary column (100% dimethylpolysiloxane, TC-1, GL Science Co., Tokyo, Japan, 30 m × 0.25 mm, I.D., 0.25 μm in film thickness) was used in both measurements. The sample volume injected was 1 μL. Split ratio was 20. Helium was used as carrier gas at the velocity of 47.0 cm/s. Column temperature was programmed at 180°C for 1 min, raised at a rate of 2°C/min to 280°C, and then kept at the temperature for 30 min. Temperature of injection port and detector was set at 250°C and 300°C, respectively.

The thioethylated monomeric products were identified by the MS data referring to the previous literature.[14] M1; MS m/z 418 (M^+, 1), 271 (9), 270 (20), 269 (100), 235 (9), 75 (10), 73 (12). M2; MS m/z 300 (M^+, 3), 212 (13), 211 (100), 178 (15). M3; MS m/z 358 (M^+, 12), 298 (9), 297 (33), 237 (13), 236 (7), 235 (32), 209 (5), 205 (5), 192 (6), 131 (6), 77 (5), 75 (100), 73 (20), 59 (5). M4; MS m/z 297 (6), 296 (M^+, 25), 281 (5), 236 (22), 235 (100), 206 (5), 205 (23), 204 (32), 189 (7), 131 (64), 115 (6), 103 (17), 75 (7), 73 (41), 59 (7). M5; MS m/z 358 (12), 298 (9), 297 (33), 237 (13), 236 (7), 235 (32), 209 (5), 192 (6), 131 (6), 75 (100), 73 (20), 59 (5). M6; MS m/z 344 (M^+, 9), 222 (10), 209 (19), 192 (7), 179 (6), 137 (9), 136 (7), 135 (100), 73 (20). M7; MS m/z 271 (10), 270 (20), 269 (100), 239 (6), 210 (7), 209 (12), 73 (19). They were determined by peak area ratio of a product to the internal standard. They were calculated, based on an assumption that response factors of all products to the internal standard were the same value (1.5).[13] The thioacidolysis of each model compound was performed in duplicate. GC/MS analysis of its thioacidolysis products was done in five replicate, and the mean value of their peak area was used for quantification.

3-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxy-4-propylphenoxy)-propane-1-ol [as TMS derivative], a dimeric product of incomplete β-ether cleavage observed after thioacidolysis followed by desulfurization, was identified by the MS data referring to the previous literature.[17] MS m/z 491 (2.4), 490 (M^+, 5.8), 293 (2.1), 238 (4.2), 223 (4.9), 211 (4.3), 210 (18), 209 (100), 179 (9.5), 137 (2.4), 103 (6.0), 73 (31).

HPSEC Analysis of Thioacidolysis Products

An aliquot (ca. 0.8 mL) of the concentrated dichloromethane extract of conventional thioacidolysis products from CA, Compounds Ib and II was concentrated under reduced pressure to syrup, dried over phosphorus oxide (V) in a vacuum desiccator at room temperature overnight, and weighed. It was dissolved in a prescribed amount of tetrahydrofuran (THF) so that the concentration of the solution would be 5.0 mg/mL. Prior to HPSEC analysis, all samples were filtrated through a membrane filter (Whatman Co., Poly(Tetra-Fluoro Ethylene) [PTFE] type, pore size: 0.45 μm).

Compound II (100 mg) was acetylated with acetic anhydride (1.0 mL) in pyridine (1.0 mL) at room temperature for 24 h to enhance its solubility in THF. The acetylation was quenched by the addition of aqueous methanol. Acetylating reagents were azeotropically removed with toluene. Acetylated Compound II was dissolved in THF (5.0 mg/mL), filtrated through the membrane filter, and subjected to HPSEC analysis.

The HPSEC analysis was carried out on an LC system, composed of a dual plunger pump (Tosoh CCPS; Tosoh Co. Ltd., Tokyo, Japan), a column oven (Waters CHM; Waters Co. Ltd., Milford, MA, USA), a UV detector (Hitachi L-4000; Hitachi Co. Ltd., Tokyo, Japan), and a temperature controller (Waters CTM ; Waters Co., Ltd., Milford, MA, USA), and an integrator (Hitachi D-2520 GPC integrator; Hitachi Co. Ltd., Tokyo, Japan). SEC (GPC) columns (Shodex GPC KF-803L and KF-802, 300 mm × 8.0 mm, I.D., 6.0 μm particle size; Showa Denko Co. Ltd., Kawasaki, Kanagawa, Japan) were connected tandemly and used for separation. Temperatures of the column oven and the detector were set at 40°C. THF was used as eluent, at the flow rate of 0.5 mL/min. The injection volume was 20 μL. The columns were calibrated with polystyrene standards in the range of 30000 to 580. Wavelength of detection was set at 280 nm.

Desulfurization as a Post Treatment of Thioacidolysis[15]

Conventional thioacidolysis of Compound II was conducted under the same condition as described in the previous section. The resulting dichloromethane extract was concentrated under reduced pressure to a volume of 1.0 mL.

To an aliquot (ca. 0.8 mL) of the concentrated dichloromethane extract, Raney nickel (slurry, 5 mL) and 1,4-dioxane (5.0 mL) was added. The test tube was sealed and the reaction was initiated by heating the mixture at 50°C in an oil bath, kept at the temperature for 4 h, and then cooled on an ice bath. The reaction was quenched by the addition of Milli-Q water (5.0 mL). The resulting mixture was neutralized with 1.0 M hydrochloric acid to adjust the pH to 3.0. The mixture was extracted with dichloromethane (3 × 5 mL) and the organic layer was dried over sodium sulfate. The dichloromethane extract was concentrated under reduced pressure to a volume of 0.3 mL. An aliquot (30 μL) of it was subjected to TMS derivatization followed by GC/MS analysis (details shown in the previous section). The reminder (ca. 0.3 mL) was characterized by ultra-performance liquid chromatography/time-of-flight mass spectrometry (UPLC/TOF-MS).

UPLC/TOF-MS Analysis of Desulfurized Thioacidolysis Products

The dichloromethane extract (ca. 0.3 mL) of the desulfurized thioacidolysis products from Compound II was concentrated under reduced pressure to syrup, dried over phosphorus oxide (V) in a vacuum desiccator at room temperature overnight, and weighed (2.1 mg). It was dissolved in 42.0 mL of methanol to serve UPLC/TOF-MS analysis (50 mg/L). Prior to the analysis, the sample was filtrated through a membrane filter (Whatman Co., Poly(Tetra-Fluoro Ethylene) [PTFE] type, pore size: 0.45 μm). No derivatization was conducted before the analysis.

The UPLC/TOF-MS analysis was performed using Waters Acquity$^{\text{TM}}$ Ultra-Performance LC system (Waters Co. Ltd., Milford, MA, USA) equipped with a time-of-flight mass spectrometer.

Separation was achieved using a reverse-phase column (Waters ACQUITY UPLC BEH C18 column, 100 mm × 2.1 mm, I.D., 1.7 μm particle size; Waters Co. Ltd., Milford, MA, USA), with a mobile phase flow rate of 0.30 mL/min. The mobile phase contained water/methanol 20/80 v/v (A) and 100% methanol (B), both of which contained 0.05% acetic acid. Gradient elution was employed starting at 100% (A), held for 0.2 min, then rising linearly to 100% (B) over 6.8 min, held for 2.1 min before being returned to the original conditions and re-equilibrated for 0.2 min, giving a total cycle time of 10 min. The injection volume was 3.0 μL.

An orthogonal acceleration time-of-flight mass spectrometer (TOF-MS) with an electrospray ionization interface was used (Waters LCT Premier$^{\text{TM}}$ XE; Waters Co. Ltd., Milford, MA, USA). Data acquisition was performed using negative ionization mode over a mass range of m/z 50 to 1000 in W-mode. Leucine enkephalin was used as the Lock Spray calibrant. Accurate masses used for correction was [M-H] m/z 554.2615.

RESULTS AND DISCUSSION

Thioacidolysis Under the Conventional Condition

A β-O-4 type artificial lignin polymer (Compound II), with number-average molecular mass of 3390 (DPn = 12.1) and weight-average molecular mass of 6990 (DPw = 24.9), was prepared.[18] The molecular mass of the artificial lignin polymer was comparable to those of isolated lignins,[18] suggesting that

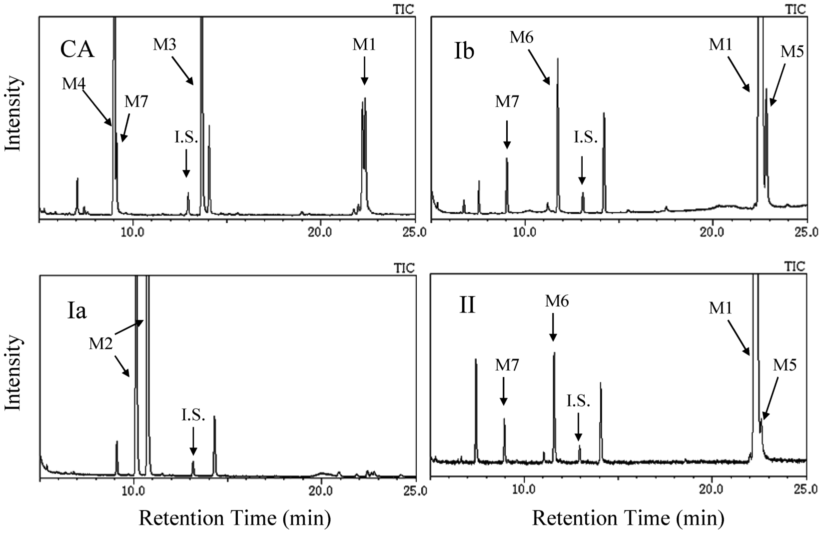


Figure 2. Total ion chromatograms of thioacidolysis products obtained by GC/MS analyses. M1–M7 denote monomeric products identified (structures shown in Figure 3). I.S.: internal standard.

the following thioacidolysis data would be applicable to characterize isolated lignins.

A monomeric lignin model (CA), dimeric lignin models (Compounds Ia and Ib), and a polymeric lignin model (Compound II) were subjected to the conventional thioacidolysis. Figure 2 shows total ion chromatograms of thioacidolysis products from the model compounds. The chromatogram of thioacidolysis products from Compound II was similar to that from Compound Ib. As expected, the β-O-4 bonds of the polymeric model were cleaved in almost the same manner as those of the dimeric model.

The chemical structures of thioethylated monomeric products were identified from mass number and retention time by referring to the literature,[14] as illustrated in Figure 3. The area of the identified peaks was summed up, and the total yields of the monomeric products by thioacidolysis reaction were estimated by the correction with the response factor (1.5) based on the area of internal standard (Table 1). Monomer yield from Compound Ib looks somewhat strange (106% as a mean value of duplicate experimental data of 104.5%

Table 1. Obtained yields of thioacidolysis monomers

	CA	Ia	Ib	II
Yield (%)	92	93	106	74

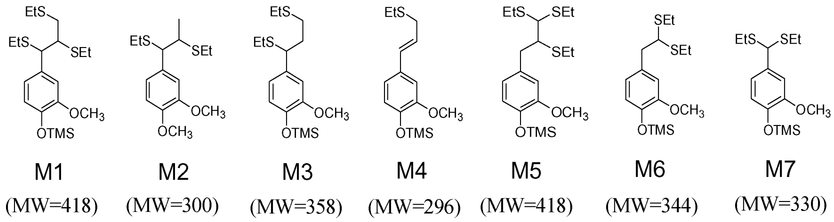


Figure 3. Structures of thioacidolysis monomeric products identified by GC/MS analyses. MW stands for molecular weight.

and 107.7%). The possible explanation for this kind of experimental error was as follows: an assumption that all identified peaks have the same response factor (1.5), and too small an amount of internal standard added in this experiment (Figure 2). However, taking the experimental error into consideration, the monomeric products from Compounds Ia and Ib were quantitatively recovered. Although the monomer yield from CA was slightly lower than those of Compounds Ia and Ib, CA was also derivatized stoichiometrically by thioacidolysis. However, the obtained yield of monomeric products from Compound II accounted only for 74% of the theoretical yield. Possible explanation for this result would be the following: (1) incomplete scission of *β*-*O*-4 bonds in the lignin model polymer under the conventional thioacidolysis conditions and (2) secondary reactions like repolymerization and condensation reaction, or both.

To estimate the degree of depolymerization of Compound II by conventional thioacidolysis, the thioacidolysis products from the model compounds were analyzed comparatively by HPSEC (Figure 4). Apparently, Compound Ib gave a single peak at the low molecular mass region that corresponds to a monomeric product, but not an oligomeric product. This result suggests that *β*-*O*-4 linkages in the dimer lignin model compound were cleaved completely without secondary polymerization reaction. Therefore, one possibility that thioacidolysis products combine together to generate higher molecular-mass compounds by secondary reaction during thioacidolysis can be eliminated. CA yielded a sharp peak and a shoulder: they were observed at the lower molecular mass region than the main peak from Compound Ib. To the contrary, Compound II provided not only a single peak at the lower molecular mass region as Compound Ib, but it also gave some shoulders at the higher molecular mass region corresponding to oligomeric thioacidolysis products.

Therefore, it is clear from our results that thioacidolysis did not cleave *β*-*O*-4 linkages in Compound II quantitatively. To generalize our observation, thioacidolysis under the conventional workup condition is likely not to achieve complete cleavage of *β*-O-4 bonds in the polymer network of lignin and its related materials.

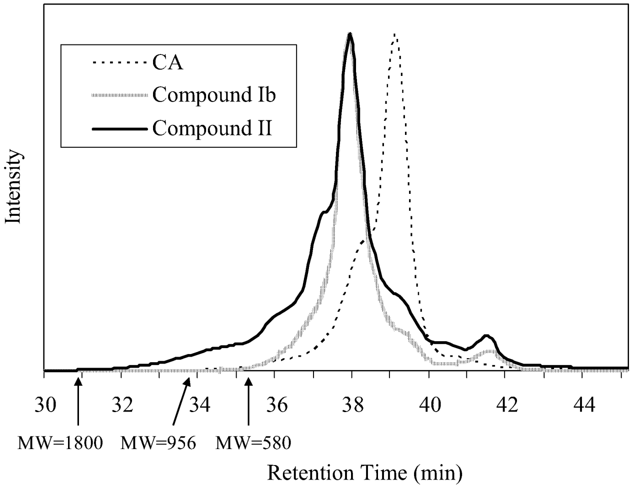


Figure 4. High performance size exclusion chromatograms (HPSEC) of thioacidolysis products from CA, Compounds Ib and II. MW and arrow indicate molecular mass calibrated by polystyrene standards.

Thioacidolysis Followed by Desulfurization

Desulfurized thioacidolysis products from Compound II were analyzed by GC/MS (Figure 5). A dimeric thioacidolysis product of guaiacyglycerol-β-guaiacyl ether type, namely, 3-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxy-

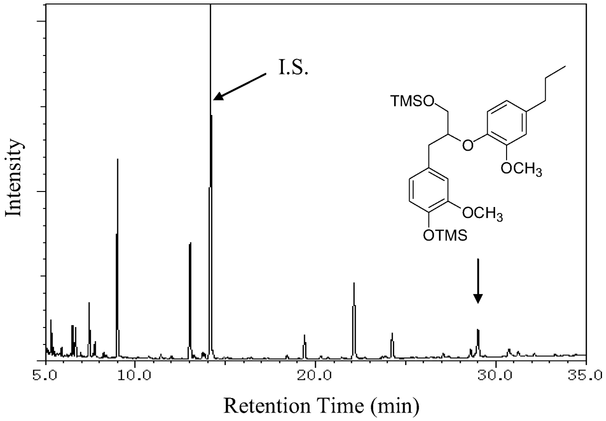


Figure 5. Total ion chromatogram of desulfurized, thioacidolysis products from artificial lignin polymer (Compound II) obtained by GC/MS analysis. I.S.: internal standard.

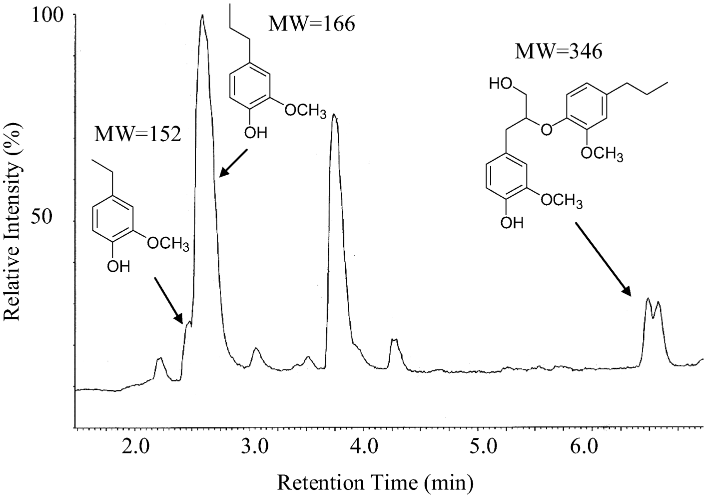


Figure 6. Total ion chromatogram of desulfurized, thioacidolysis products from compound II detected by UPLC/TOF-MS analysis. MW represents molecular weight.

4-propylphenoxy)-propane-1-ol, was identified by referring to the data of its mass fragmentation shown in the previous publication by Ralph and Grabber.[17] Although they did not derivatize the thioacidolysis products with BSTFA prior to GC/MS analysis, our result (particularly, base ion peak [mono-TMS ion; m/z 209] and parent ion peak [di-TMS ion; m/z 490] of the dimer) was in good agreement with theirs (base ion peak [m/z 137] and parent ion peak [m/z 346]). Figure 5 shows that this type of thioacidolysis product originating from incomplete cleavage of β-ether is not negligible if the frequency of β-O-4 bonds are to be discussed quantitatively by the use of thioacidolysis, although the abundance of the dimeric product is relatively small.

The authors also analyzed the desulfurized thioacidolysis products from Compound II by UPLC/TOF-MS (Figure 6). The dimeric product of guaiacyglycerol-β-guaiacyl ether type was confirmed by the use of high resolution mass chromatography with its parent ion m/z 346.1780, overlapping the total ion chromatogram. Again, it was clearly shown that thioacidolysis would probably lead to incomplete cleavage of β-ether bonds of β-O-4 type polymer.

Attempts to Improve the Thioacidolysis Monomer Yields

Effects of the reaction time and the concentration of ethanthiol on the reactivity of thioacidolysis were investigated to see if the yields of thioacidolysis products from Compound II could be improved (Table 2). Obviously, prolonged reaction time did not increase the yields of thioacidolysis products (Entries

Table 2. Effects of reaction time and concentration of thioacidolysis reagent on monomer yields

Entry	Time (h)	Ethanethiol (mmol)	Yield (%)
1	4	12	74
2	8	12	69
3	24	12	72
4	4	24	76
5	24	24	77

1–3). Increased concentration of ethanthiol did not significantly increase the monomer yields (Entries 1 and 4, or Entries 3 and 5). It seems that neither prolonged reaction time nor increased concentration of ethanethiol gave thioacidolysis monomers at quantitative yield. This result shows additional evidence that thioacidolysis does not cleave all the β-O-4 bonds in the artificial lignin polymer.

CONCLUSIONS

This study first demonstrated the reaction efficiency of thioacidolysis for cleavage of β-*O*-4 interunitary linkages, using β-*O*-4 type artificial lignin model polymer. Quantitative cleavage of β-*O*-4 linkages in the lignin model polymer was not observed under the conventional thioaciodolysis condition, although the thioacidolysis monomers from monomeric and dimeric lignin models were quantitatively obtained. The efficiency of thioacidolysis reaction for compound II was not improved by either the prolonged reaction time or increased concentration of the thioacidolysis reagent. It was deduced from these experiments that further improvement of thioacidolysis technique to increase the monomer yields was quite difficult to achieve in our lab. Therefore, this reaction efficiency of thioacidolysis to the polymeric lignin model is noteworthy, if thioacidolysis is to be applied to the quantitative estimation of the frequency of β-*O*-4 interunitary linkages in lignin preparations.

REFERENCES

1. Leopold, B. Aromatic keto- and hydroxyl-polyethers as lignin models. III. Acta Chem. Scand. **1950**, *4*, 1523–1537.
2. Larsson, S.; Miksche, G.E. Gas chromatographic analysis of lignin oxidation products. The diphenyl ether linkage in lignin. Acta Chem. Scand. **1967**, *21*(7), 1970–1971.
3. Matsukura, M.; Sakakibara, A. Hydrogenolysis of protolignin. V. Isolation of some dimeric compounds with carbon to carbon linkage. Mokuzai Gakkaishi **1973**, *19*(3), 131–135.

4. Lundquist, K.; Kirk, T.K. Acid degradation of lignin. Analysis of lignin acidolysis products by gas chromatography, using trimethylsilyl derivatives. Acta Chem. Scand. **1971**, *25*(3), 889–894.
5. Lapierre, C.; Pollet, B.; Rolando, C. New insights into the molecular architecture of hardwood lignins by chemical degradative methods. Res. Chem. Intermed. **1995**, *21*(3–5), 397–412.
6. Matsumoto, Y.; Ishizu, A.; Nakano, J. Study on chemical structure of lignin by ozonation. Holzforschung **1986**, *40*(Suppl.), 81–85.
7. Akiyama, T.; Magara, K.; Matsumoto, Y.; Meshitsuka, G.; Ishizu, A.; Lundquist, K. Proof of the presence of racemic forms of arylglycerol-β-aryl ether structure in lignin: Studies on the stereo structure of lignin by ozonation. J. Wood Sci. **2000**, *46*(5), 414–415.
8. Lu, F.; Ralph, J. Derivatization followed by reductive cleavage (DFRC Method), a new method for lignin analysis: Protocol for Analysis of DFRC Monomers. J. Agric. Food Chem. **1997**, *45*(7), 2590–2592.
9. Lu, F.; Ralph, J. DFRC method for lignin analysis. 1. New method for β-aryl ether cleavage: Lignin model studies. J. Agric. Food Chem. **1997**, *45*(12), 4655–4660.
10. Katahira, R.; Ujihara, M.; Nakatsubo, F. A novel selective cleavage method for β-O-4 substructure in lignins named TIZ method. I. Degradation of guaiacyl and syringyl models. J. Wood Chem. Technol. **2003**, *23*(1), 71–87.
11. Lapierre, C.; Monties, B.; Rolando, C. Thioacidolysis of lignin: Comparison with acidolysis. J. Wood Chem. Technol. **1985**, *5*(2), 277–292.
12. Lapierre, C.; Monties, B.; Rolando, C. Thioacidolysis of poplar lignins: Identification of monomeric syringyl products and characterization of guaiacyl-syringyl lignin fraction. Holzforschung **1986**, *40*(2), 113–118.
13. Lapierre, C.; Monties, B.; Rolando, C. Preparative thioacidolysis of spruce lignin: Isolation and identification of main monomeric products. Holzforschung **1986**, *40*(1), 47–50.
14. Brunow, G.; Kilpelainen, I.; Lapierre, C.; Lundquist, K.; Simola, L.K.; Lemmetyinen, J. The chemical structure of extracellular lignin released by cultures of *Picea abies*. Phytochemistry **1993**, *32*(4), 845–850.
15. Lapierre, C.; Pollet, B.; Monties, B; Rolando, C. Thioacidolysis of spruce lignin: Gas chromatography-mass spectroscopy analysis of the main dimmers recovered after Raney nickel desulphuration. Holzforschung **1991**, *45*(1), 61–68.
16. Lapierre, C.; Roland, C. Thioacidolysis of pre-methylated lignin samples from pine compression and poplar woods. Holzforschung **1988**, *42*(1), 1–4.
17. Ralph, J.; Grabber, J.H. Dimeric β-ether thioacidolysis products resulting from incomplete ether cleavage. Holzforschung **1996**, *50*(5), 425–428.
18. Kishimoto, T.; Uraki, Y.; Ubukata, M. Chemical synthesis of β-O-4 type artificial lignin. Org. Biomol. Chem. **2006**, *4*(7), 1343–1347.
19. Kishimoto, T.; Sano, Y. Delignification mechanism during high-boiling solvent pulping. V. Reaction of non-phenolic β-O-4 model compounds in the presence and absence of glucose. J. Wood Chem. Technol. **2003**, *23*(3&4), 279–292.
20. Adler, E.; Lindgren, B.O.; Saeldén, U. The beta-guaiacyl ether of alpha-veratryl glycerol as a lignin model. Sven. Papperstidn. **1952**, *55*, 245–254.

21. Matsumoto, Y.; Ishidzu, A.; Iiyama, K.; Nakano, J. Selective cleavage of arylglycerol-β-aryl ether type structures in lignin. II. Determination of the arylglycerol-β-aryl ether type structure in milled wood lignin of spruce. Mokuzai Gakkaishi **1982**, *28*(4), 249–254.
22. Nakatsubo, F.; Sato K.; Higuchi, T. Synthesis of guaiacyl glycerol-β-guaiacyl ether. Holzforschung **1975**, *29*(5), 165–168.
23. Quideau, S.; Ralph, J. Facile large-scale synthesis of coniferyl, sinapyl, and *p*-coumaryl alcohol. J. Agrlc. Food Chem. **1992**, *40*(7), 1108–1110.