Model Experiments to Study the Inhibition Mechanism of Lignin Self-Condensation under *tert*-Butyl Alcohol and Concentrated Sulfuric Acid Conditions

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The concentrated sulfuric acid/*tert*-butyl alcohol (TBA) hydrolysis system efficiently converts lignocellulose into monosaccharides and *tert*-butyl alcohol lignin (TBL), which exhibits thermal plasticity and a high solubility in common organic solvents. However, the reaction mechanism and the TBL chemical structure remain unclear. In this study, 2-methoxy-4-methylphenol (creosol, 4-methylguaiacol) was used as a model compound for the guaiacyl ring of lignin, and was treated with the concentrated sulfuric acid/TBA system to elucidate the chemical structure of TBL. At the beginning of this reaction, TBA rapidly reacted with 4-methylguaiacol to produce two main products. These products were fractionated using high-performance liquid chromatography and analyzed using nuclear magnetic resonance (NMR) spectroscopy to determine their chemical structures. The NMR spectra of the products revealed that the *tert*-butyl group was incorporated into either the *ortho* position of the phenolic OH group or the *para* position of the methoxyl group of 4-methylguaiacol. The former product was stable, whereas the latter gradually decomposed in the presence of sulfuric acid. This model experiment suggests that the consumption of anionic species on the lignin aromatic ring could be effective in preventing lignin self-condensation between the aromatic ring and the benzyl position of lignin under sulfuric acid treatment.

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INTRODUCTION

The two-step sulfuric acid saccharification of lignocellulose has been conventionally used to obtain high yields of monosaccharides from various types of lignocellulose (Liu *et al.* 2012; Wijaya *et al.* 2014; Zhou *et al.* 2021). Compared with other bioconversion processes using alkali, organic solvents, ionic liquids, or deep eutectic solvents (Nair *et al.* 2023; Norfarhana *et al.* 2024; Xiao *et al.* 2024; Wang *et al*. 2025), one of the advantages of this process is its high saccharification efficiency, in which cellulose and hemicellulose are hydrolyzed to selectively produce monosaccharides. These obtained monosaccharides can subsequently be converted into sustainable biofuels and biochemicals (Menon and Rao 2012; Tse *et al.* 2021; Pereira *et al.* 2025). In contrast, the chemical structure of lignin becomes highly condensed in concentrated sulfuric acid, producing low-value sulfuric acid lignin with a low chemical reactivity, a lack of thermal plasticity, and poor solubility in common organic solvents (Matsushita *et al.* 2009; Takada *et al.* 2023). Therefore, to fully utilize all lignocellulosic components, including not only the polysaccharides but also the lignin component, it is necessary to prevent the condensation of lignin during concentrated sulfuric acid treatment.

The mechanism of lignin condensation in concentrated sulfuric acid has been extensively studied over the last half century (Leary *et al.* 1986; Yasuda and Ota 1987). First, the hydroxyl group at the α-position (*i.e.*, the benzyl position) is eliminated to give a benzyl cation. Subsequently, electron donation from the methoxy group of another lignin molecule produces an anion at the C6 position, leading to an electrophilic aromatic substitution reaction and C–C bond formation between the α and C6 positions (Fig. 1a) (Yasuda *et al.* 1981). Therefore, the intra- and intermolecular self-condensation of lignin produces a highly branched and complex structure of sulfuric acid lignin. To avoid this issue Shiraki *et al.* (2021) developed a method to suppress the self-condensation of lignin while maintaining a high yield of monosaccharides, wherein *tert*-butyl alcohol (TBA) was added during the two-step sulfuric acid saccharification of lignocellulose. In this process, it was assumed that the *tert*-carbocation produced by elimination of the hydroxyl group from TBA is introduced into the aromatic ring of the lignin (Fig. 1b), consuming the aromatic anion which is the reaction site for intra- and intermolecular self-condensation of lignin. Furthermore, the incorporated *tert*-butyl group may then contribute to suppression of the self-condensation of lignin owing to its steric hindrance. Hence, the resultant *tert*-butyl alcohol lignin (TBL) is thermally fusible and soluble in common organic solvents, both of which are desirable properties in the context of lignin-derived carbon materials. However, the chemical structure of TBL remains unclear due to its complex nature and high molecular weight. Thus, to achieve the functional application of TBL based on its molecular design, it is necessary to understand both its chemical structure and the reaction mechanism by which the lignin aromatic ring react with TBA.

In this study, a phenolic monomer bearing guaiacyl ring is treated with the concentrated sulfuric acid/TBA system to identify representative chemical structure of the TBL. For this purpose, 2-methoxy-4-methylphenol (creosol, 4-methylguaiacol, Fig. 1c) is employed as the lignin monomer model compound. The obtained products are fractionated using high-performance liquid chromatography (HPLC), and are subsequently analyzed by nuclear magnetic resonance spectroscopy (NMR) to determine their chemical structures. It is proposed that this model experiment will provide knowledge regarding the chemical structure of TBL, while also yielding a unique strategy for the prevention of lignin self-condensation under concentrated sulfuric acid conditions.

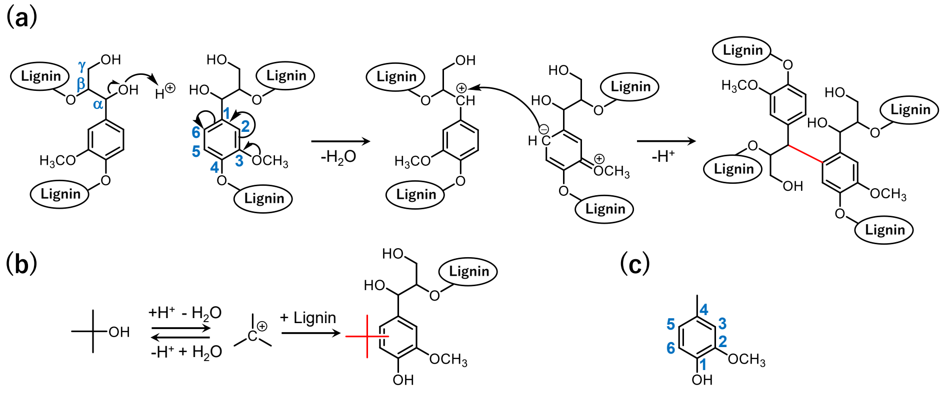


Fig. 1. (a) Reaction mechanism for the self-condensation of lignin in concentrated sulfuric acid; (b) Predicted mechanism for the reaction of TBA with lignin in concentrated sulfuric acid; (c) Chemical structure of 4-methylguaiacol.

EXPERIMENTAL

Materials

4-Methylguaiacol was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), TBA was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan), and the 95 wt.% sulfuric acid was purchased from Wako Pure Chemical Co. (Osaka, Japan).

Model Experiment for Concentrated Sulfuric Acid/TBA Treatment

In a 30 mL beaker, 4-methylguaiacol (0.25 g), deionized water (0.86 g), and TBA (8.27 g) were mixed. Concentrated sulfuric acid (95 wt%, 9.00 g) was then added dropwise to the mixture over 10 min under magnetic stirring in an ice bath, and the resulting mixture was diluted with a saturated saline solution (250 g). The combined solution was extracted twice with diethyl ether (250 mL). A diethyl ether solution (1 mL) containing 4-ethylguaiacol (0.3 g/mL), which has a similar structure to 4-methylguaiacol, was added to the extract as an internal standard (IS). The solvent was then dried over anhydrous Na_2SO_4, filtered and distilled using an evaporator to obtain an oily product. This product was subjected to gas chromatography–mass spectrometry (GC/MS) using a GCMS-QP2010 system (Shimadzu, Kyoto, Japan) equipped with a DB-5 column (30 m × 0.25 mm id, 1.00 µm film thickness; Agilent technologies, Santa Clara, CA, USA). The sample (1 µL) was injected in the split mode at an injection temperature of 250 °C. The column temperature was set at 40 °C for 2 min, and then increased to 270 °C at a rate of 20 °C/min. Helium was used as a carrier gas at a flow rate of 1.70 mL/min. The mass spectra were recorded in the electron impact ionization mode (70 eV).

Product Fractionation

The product obtained by concentrated sulfuric acid/TBA treatment was fractionated using an HPLC system (Shimadzu, Kyoto, Japan) equipped with a Cadenza 5CD-C18 column (250 × 20 mm id; Imtakt, Kyoto, Japan) and an SPD-M20A PDA detector (Shimadzu, Kyoto, Japan). A mixture of methanol and distilled water (8:2, v/v) was used as an eluent at a flow rate of 15 mL/min. After fractionation, an aliquot (40 mL) of saturated saline was added to fractions A and B, and the solutions were extracted three times with hexane (60 mL). The solvent was then dried over anhydrous Na_2SO_4, filtered and distilled using an evaporator. The purified products were dissolved in pyridine and subjected to GC/MS.

NMR Analysis of the Fractionated Products

The chemical structures of the purified products were determined using a 500 MHz NMR spectrometer (JNM-ECZ500R, JEOL, Tokyo, Japan). Purified products A (2.0 mg) and B (3.6 mg) were dissolved in chloroform-*d* containing 0.03% TMS (600 µL). The NMR signals of the products were assigned based on the one-dimensional ^1H and ^{13}C NMR spectra, along with the two-dimensional (2D) ^1H-^{13}C HSQC (heteronuclear single quantum coherence) and ^1H-^{13}C HMBC (heteronuclear multiple bond correlation) measured at 303 K. The 2D HMBC NMR spectra were based on a spectral width of 9 ppm on the ^1H axis (2048 data points) and 170 ppm on the ^{13}C axis (256 data points). A total of four scans were performed.

Influence of the TBA Amount and Reaction Time on the Product Yields

In a 30 mL beaker, deionized water and TBA were added to 4-methylguaiacol (0.25 g) according to the quantities listed in Table 1. Concentrated sulfuric acid (95 wt.%, 9.00 g) was added dropwise to the mixture over 10 min under magnetic stirring in an ice bath. The reaction vessel was then transferred to a hot water bath at 40 °C and stirred for 0, 10, or 60 min. After the desired time, the reactants were diluted with a saturated saline solution (250 g), and extracted twice with diethyl ether. An aliquot (1.0 mL) of the IS in diethyl ether (0.3 g/mL) was then added to the extract, and the sample was diluted to 250 mL using diethyl ether. After subsequent drying over anhydrous Na_2SO_4, the sample was analyzed using GC/MS.

Table 1. TBA and Water Quantities used in the Experiments

	TBA (mol)	Water (mol)	Total amounts of TBA and water (mol)
Entry 1	0.11	0.05	0.16
Entry 2	0.08	0.08	0.16
Entry 3	0.06	0.10	0.16
Entry 4	0.03	0.13	0.16
Entry 5	0.01	0.15	0.16

RESULTS AND DISCUSSION

Evaluation of the Products Obtained Following Concentrated Sulfuric Acid/TBA Treatment

Following reaction of the lignin model compound, 4-methylguaiacol, with concentrated sulfuric acid and TBA, the obtained products were analyzed by GC/MS, as presented in Fig. 2a. The peaks observed at retention times (RTs) of 4.2–4.4, 7.7–7.9, 8.0–8.4, 10.2, and 10.4–10.9 min corresponded to the condensation products of TBA, such as isobutylene, di-isobutylene, 1,1-dineopentylethylene, and 2,2,4,6,6-pentamethyl-3-heptene. As previously reported, isobutylene is produced by the elimination of a hydroxyl group from TBA, followed by the formation of a double bond, as illustrated in Fig. 2b (Bothe *et al.* 2006). Additionally, dimers and trimers of isobutylene were produced via condensation of the obtained isobutylene with TBA.

Additionally, at RTs of 11.8 and 12.5 min, the main products of the reaction, namely compounds A and B, were detected. The molecular ion peak at *m/z* 194 (Fig. 2c) in each case suggests the presence of 5-(*tert*-butyl)-4-methylguaiacol (5-TBM) and 6-(*tert*-butyl)-4-methylguaiacol (6-TBM) produced by the incorporation of TBA into the aromatic ring of 4-methylguaiacol (Fig. 2d). Furthermore, the largest fragment ion peak at *m*/*z* 179 suggested the chemical structures of 5- and 6-TBMs with demethylation at the C4 carbon. The fragment ion peak at *m*/*z* 91 suggested the TBM structures resulting from the detachment of *tert*-butyl, methyl, and methoxy groups. Hence, compounds A and B were found to be 5-TBM or 6-TBM. However, the structural differences between 5-TBM and 6-TBM were not distinguishable in the MS results, and so further structural analysis was required to clearly define each peak.

Fractionation and Structural Analysis of the Products

The products obtained in the model experiment were fractionated using HPLC, wherein the chromatogram presented in Fig. 3a confirms the successful separation of compounds A and B. Furthermore, the high purities of these fractionated compounds were confirmed by the GC chromatogram shown in Fig. 3b.

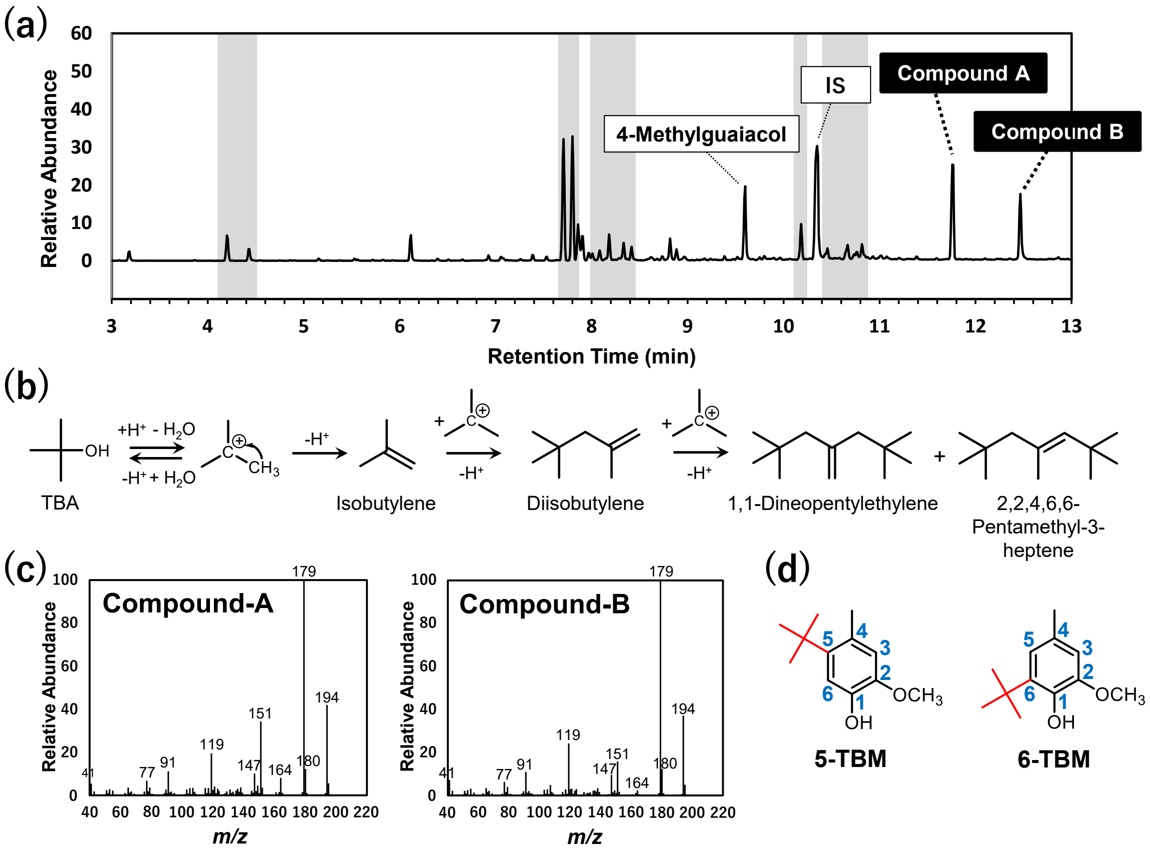


Fig. 2. (a) GC/MS chromatogram of the products obtained by reaction of 4-methylguaiacol with concentrated sulfuric acid and TBA. The peaks indicated by gray bands correspond to the condensation products of TBA. (b) Reaction mechanism of TBA condensation in concentrated sulfuric acid. (c) MS spectra of compounds A and B. (d) Chemical structures of 5-TBM and 6-TBM.

The chemical structures of the purified compounds (A and B) were subsequently determined by NMR analysis, and their ^{1}H and ^{13}C NMR spectra are shown in Fig. 4. These spectra contained signals at δ_C/δ_H 29.52–30.98/1.37–1.39 ppm and 21.47–22.69/2.28–2.46 ppm corresponding to the *tert*-butyl CH_3 and the C4-methyl groups of the 5-TMB and 6-TBM structures, respectively. Additionally, the NMR signals at δ_C/δ_H 109.44–146.53/6.58–6.98 ppm and 55.89–56.19/3.85–3.86 ppm were assigned to aromatic rings and C2-methoxy groups of these two compounds, respectively. Figure 5 shows the HMBC spectra of compounds A and B. As indicated, HMBC correlations between the carbon atom of the C4-methyl group and the protons of the aromatic ring were observed in both HMBC spectra at δ_C/δ_H 21.47–22.69/6.58–6.69 ppm, although the spectrum of compound A contained two signals and this region while that of compound B contained one signal.

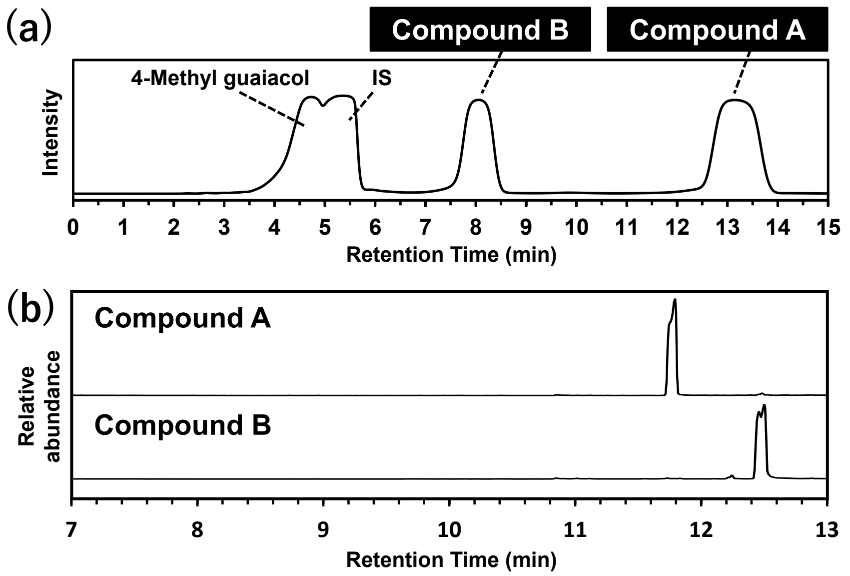


Fig. 3. (a) HPLC chromatogram of the products obtained following the sulfuric acid/TBA treatment of 4-methylguaiacol. (b) GC/MS chromatograms of purified compounds A and B.

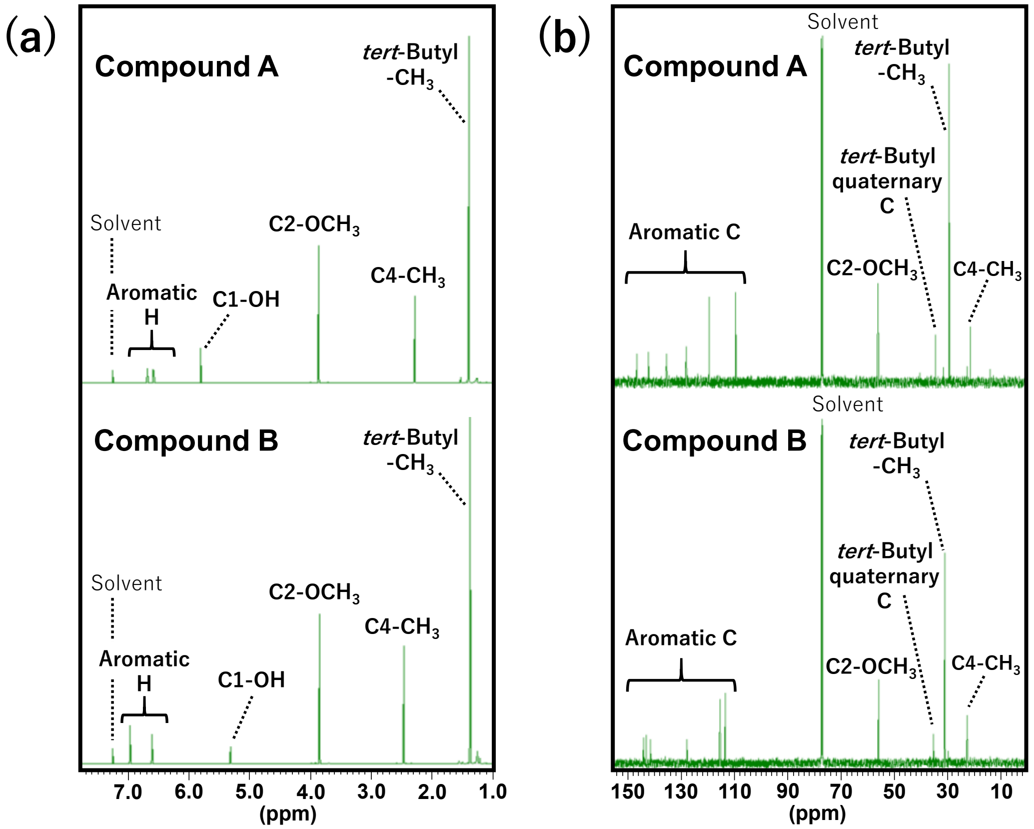
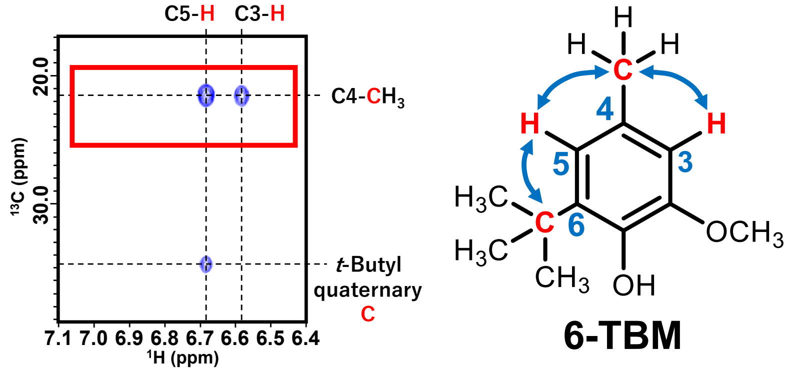


Fig. 4. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of compounds A and B.

This difference indicates that the carbon atom of the C4-methyl group in compound A is involved in an HMBC correlation with both the H-3 and H-5 positions of the aromatic ring, whereas the corresponding carbon atom in compound B is involved in only a single HMBC correlation with the H-3 position, as illustrated in Fig. 5. These observations allowed compounds A and B to be identified as 6-TBM and 5-TBM, respectively.

Moreover, HBMC correlations between the quaternary carbon atom of the *tert*-butyl group and the aromatic protons (δ_C/δ_H 34.63–35.24/6.69–6.98 ppm) further confirmed the successful incorporation of TBA into the aromatic ring of 4-methylguaiacol. The chemical shifts obtained for 5-TBM and 6-TBM are presented in Table 2.

(a) **Compound A**



(b) **Compound B**

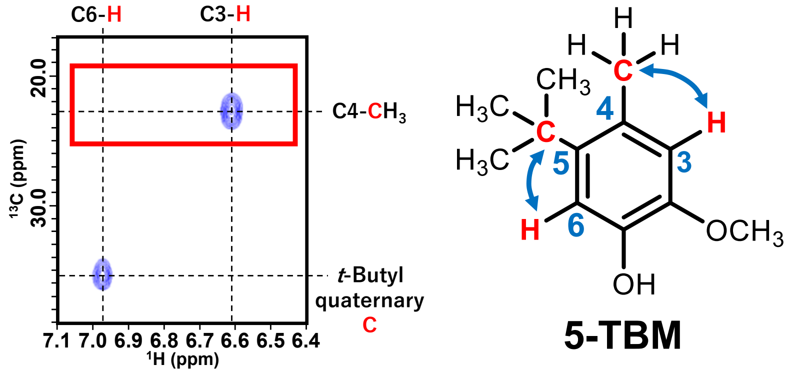


Fig. 5. HMBC spectra and determined chemical structures of (a) compound A (6-TBM), and (b) compound B (5-TBM). The red frames indicate HMBC correlations between the carbon atom of the C4-methyl group and the protons of the aromatic ring.

Table 2. Chemical Shifts Obtained from the ^{1}H and ^{13}C NMR Spectra of 5-TBM and 6-TBM (CDCl$_3$)

	5-TBM		6-TBM	
Position	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)	^{13}C (ppm)
C1	-	142.70	-	141.99
C2	-	143.82	-	146.53
C3	6.61	115.24	6.58	109.44
C4	-	127.54	-	127.89
C5	-	141.15	6.69	119.44
C6	6.98	113.14	-	135.27
C1-OH	5.32	-	5.80	-
C2-OCH$_3$	3.85	55.89	3.86	56.19
C4-CH$_3$	2.46	22.69	2.28	21.47
t-Butyl CH$_3$	1.37	30.98	1.39	29.52
t-Butyl quaternary C	-	35.24	-	34.63

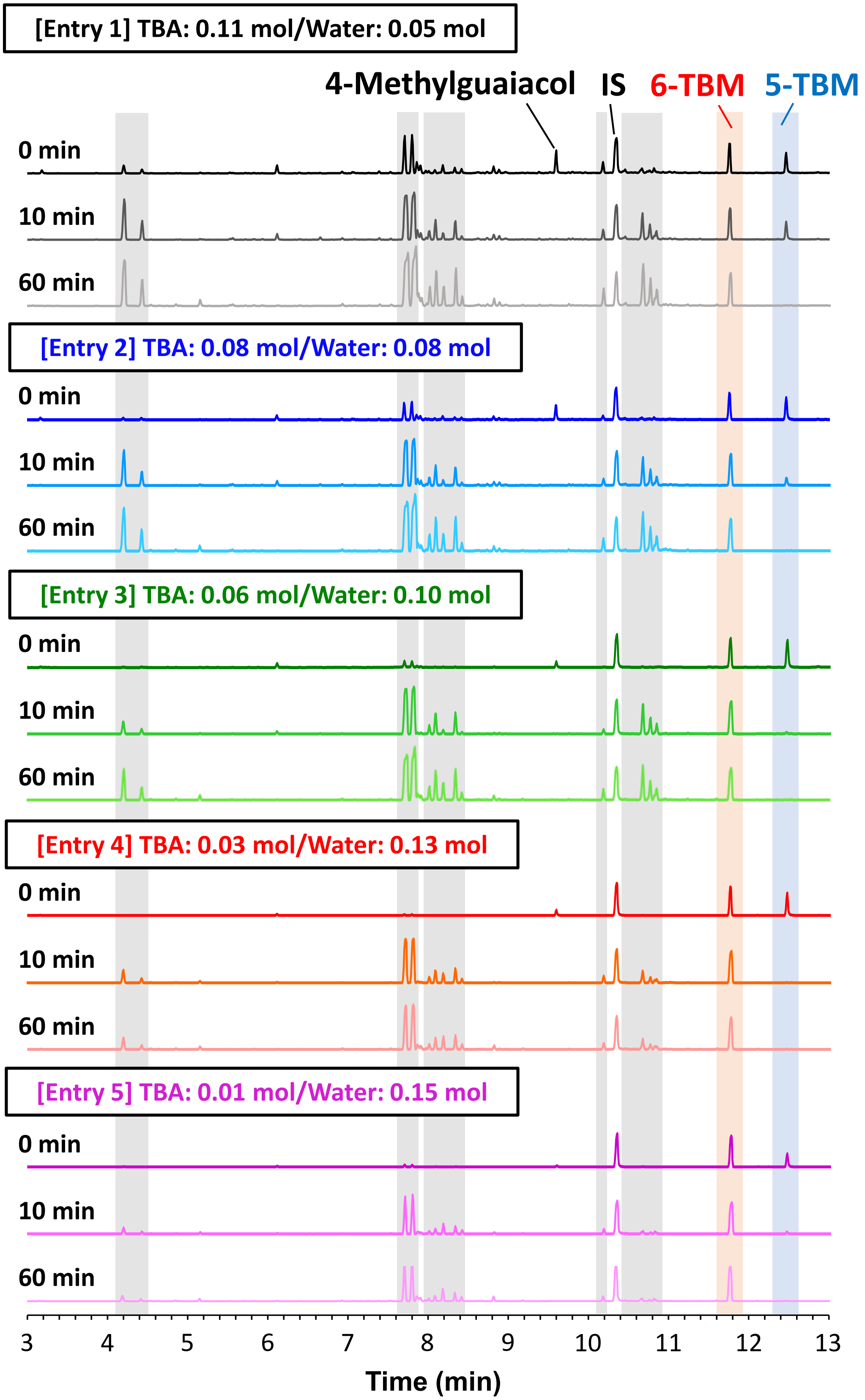


Fig. 6. GC/MS chromatograms of the products obtained following the sulfuric acid/TBA treatment of 4-methylguaiacol with different TBA amounts and over varying reaction times. The peaks indicated by gray bands correspond to the condensation products of TBA.

Effects of the TBA Amount and the Reaction Time on the Product Yields

The effects of the TBA amount and the reaction time on the model experiment performed for the concentrated sulfuric acid/TBA system were subsequently investigated. Figure 6 shows the GC/MS chromatograms of the products obtained under the different reaction conditions, while Fig. 7 shows the peak area ratios of the TBMs to the IS.

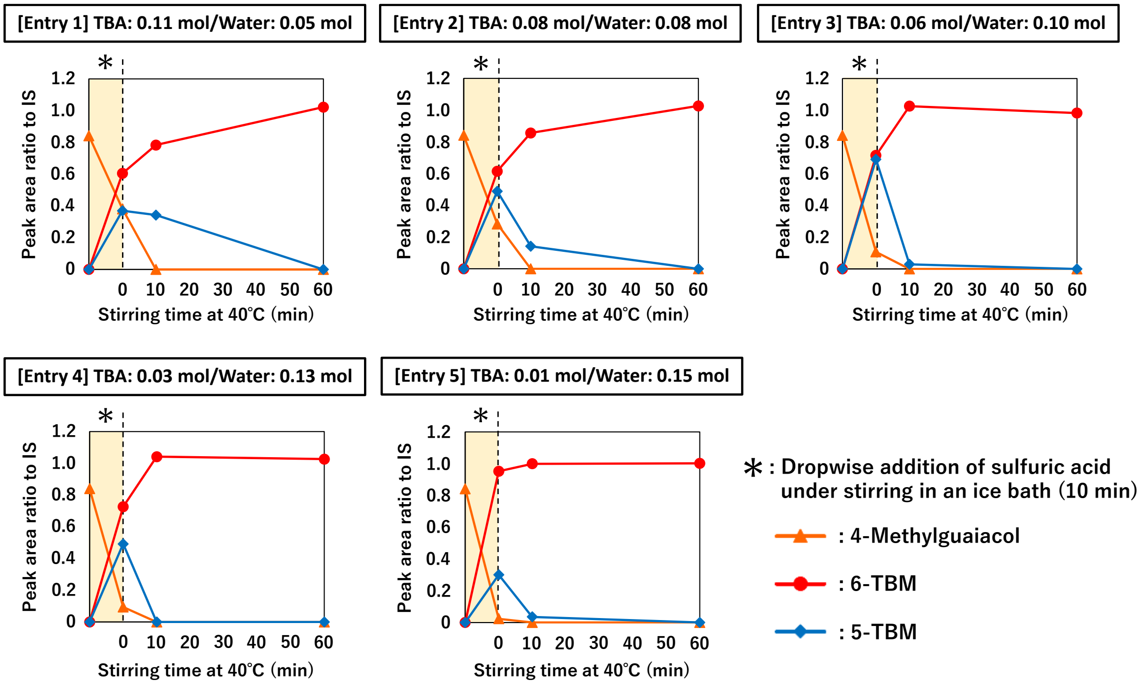


Fig. 7. GC peak area ratios of the TBMs to the IS, wherein the reaction of 4-methylguaiacol with concentrated sulfuric acid/TBA was carried out using different TBA amounts and reaction times.

The starting material, 4-methylguaiacol (RT: 9.6 min), was rapidly consumed and was not observed after stirring for 10 min. On the other hand, the products 6-TBM and 5-TBM (RTs: 11.8 and 12.5 min) began to form prior to stirring at 40 °C, indicating that the reaction progressed rapidly during the dropwise addition of sulfuric acid over 10 min. Using 0.01–0.06 mol of TBA, the production rate of 6-TBM was high, and reached a maximum after stirring for 10 min. When the reaction was performed using 0.01 mol TBA, the maximum peak area obtained for 6-TBM was similar to that obtained using 0.11 mol TBA, thereby indicating that the use of 0.01 mol TBA is sufficient for maximizing the 6-TBM yield, and suggesting that TBA preferentially reacts with 4-methylguaiacol to produce 6-TBM. Interestingly, 5-TBM was unstable and its concentration decreased during stirring. This may be explained by considering the conversion of 5-TBM into other monomeric compounds; however, further analysis is required to clarify this process. Di- and tri-isobutylenes were also identified as side products in this reaction (RTs: 4.2–4.4, 7.7–7.9, 8.0–8.4, 10.2, and 10.4–10.9 min), wherein their quantities increased upon prolonging the stirring time and increasing the TBA dosage. Based on the above results, the reaction conditions using small amount of TBA (0.01–0.06 mol) with short reaction time are effective to selectively obtain 5-TBM and 6-TBM in this model experiment.

Proposed Mechanism for Preventing Lignin Self-Condensation during Concentrated Sulfuric Acid/TBA Treatment

The model experiment performed using 4-methylguaiacol in concentrated sulfuric acid/TBA revealed that TBA was incorporated into the aromatic ring of 4-methylguaiacol, producing 5-TBM and 6-TBM as products. Based on these results, a mechanism was proposed to avoid the self-condensation of lignin during the concentrated sulfuric acid treatment of lignocellulose (Fig. 8). More specifically, in the concentrated sulfuric acid/TBA system, the *tert*-carbocation generated from TBA (rather than the lignin benzyl cation) rapidly reacts with the anionic species on the aromatic ring of lignin. Importantly, the consumption of these aromatic anions reduces the possibility of intra- and intermolecular condensation.

Although this mechanism was clearly demonstrated by the model experiment, it is additionally speculated that the bulk structure of the incorporated *tert*-butyl group may hinder interactions between lignin molecules due to its steric hindrance, thereby also reducing the possibility of lignin self-condensation. Therefore, the TBL obtained through the concentrated sulfuric acid/TBA treatment of lignocellulose would be considered to possess a structure with a low degree of condensation, therefore accounting for its desirable thermal/melting properties and its solubility in common organic solvents.

Although several previous studies have reported bioconversion systems based on the use of phenolic derivatives, formaldehyde, and 1,4-butanediol to prevent the self-condensation of lignin (Funaoka and Abe 1989; Shuai *et al.* 2016; Du *et al.* 2025; He *et al.* 2022; Li *et al.* 2024), the concentrated sulfuric acid/TBA system was found to demonstrate a unique mechanism for preventing lignin condensation.

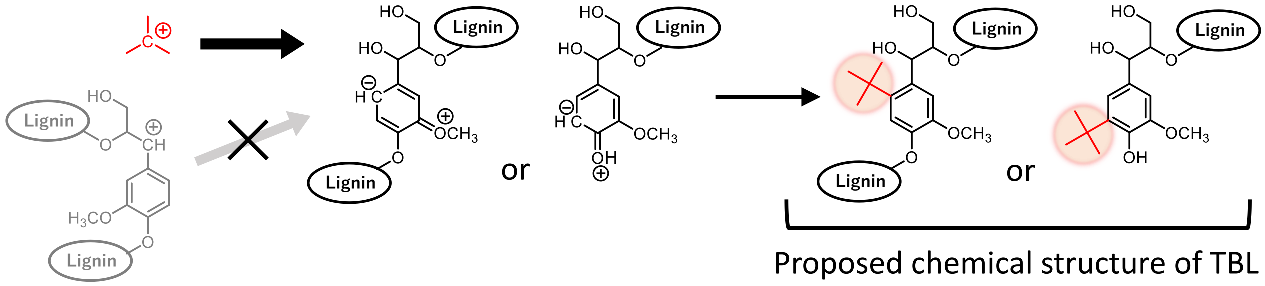


Fig. 8. Proposed mechanism for preventing lignin self-condensation in the concentrated sulfuric acid/TBA system. TBA rather than the lignin benzyl cation rapidly reacts with an anionic species on the lignin aromatic ring, producing aromatic ring possessing a *tert*-butyl group.

CONCLUSIONS

The treatment of lignocellulose with concentrated sulfuric acid/TBA yields hydrolyzed monosaccharides and TBL, a functional lignin with high solubility in common organic solvents. In this study, the chemical structure of TBL was evaluated using a 4-methylguaiacol model compound. After treatment with the concentrated sulfuric acid/TBA system, the two main products were purified by HPLC, analyzed by NMR spectroscopy, and identified as 5-TBM and 6-TBM. The production of these TBM products reached completion in the initial stages of the reaction, indicating that TBA was rapidly incorporated into the aromatic ring of lignin. This result suggests that the rapid consumption of aromatic anions could contribute to the inhibition of lignin self-condensation. In addition, steric hindrance arising from the introduced *tert*-butyl groups is also suggested to suppress lignin self-condensation. This reaction design is important for isolating low-condensation lignin and for developing its use as a new lignin-based material.

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