Tetramethylammonium Hydroxide (TMAH) Thermochemolysis of Lignin: Behavior of 4-*O*-Etherified Cinnamyl Alcohols and Aldehydes

KEN-ICHI KURODA*,† AND AKIKO NAKAGAWA-IZUMI‡

Department of Forest and Forest Products Sciences, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan, and Institute of Agricultural and Forest Engineering, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

The thermochemolytic behavior of 4-*O*-etherified cinnamyl alcohols and aldehydes in lignin was investigated in the presence of tetramethylammonium hydroxide (TMAH) (315 °C/4 s), using veratrylglycol-β-(coniferyl alcohol) ether (**1a**), veratrylglycol-β-(sinapyl alcohol) ether (**1b**), and veratrylglycol-β-(coniferyl aldehyde) ether (**2**). The methylated products were monitored with gas chromatography—mass spectrometry. Dimers **1a** and **1b** provided the coniferyl and sinapyl alcohol dimethyl ethers consisting of three isomers, respectively. Coniferyl alcohol dimethyl ether isomers were also observed in the TMAH thermochemolysis pyrolysates of a bulk dehydrogenation polymer of coniferyl alcohol and a Japanese cedar (*Cryptomeria japonica*) wood. Coniferyl aldehyde methyl ether was not provided from TMAH thermochemolyses of coniferyl aldehyde, **2**, a dehydrogenation polymer of coniferyl aldehyde, and the cedar wood. The former three provided veratryl aldehyde in a large abundance, instead of coniferyl aldehyde methyl ether. Sinapyl aldehyde provided 3,4,5-trimethoxybenzaldehyde in a large abundance and sinapyl aldehyde methyl ether in a trace abundance. The results showed that TMAH thermochemolysis is an effective tool to obtain information on cinnamyl alcohol end groups, but is not applicable to analysis of cinnamyl aldehyde end groups.

INTRODUCTION

Lignins are biopolymers connected by C—O and C—C linkages, accounting for 15—36 wt % of woody plants (*1*). They arise from an enzyme-mediated dehydrogenation of *p*-hydroxy-cinnamyl alcohols and therefore involve 4-*O*-etherified cinnamyl alcohols and aldehydes as end groups. Such end groups make a smaller contribution to the lignin polymer [e.g., 3 and 3—4% for coniferyl alcohol end groups and coniferyl aldehyde end groups, respectively, in a spruce milled wood lignin (*2*)] than β-O-4 linkages (48 and 60% of the total interunit linkages in spruce and birch milled wood lignins, respectively (*3*)]. However, the end groups are important for the evaluation of the total lignin structure because they can serve as a sensitive index for the structural change and the overall character of the lignin (*4*).

Cinnamyl alcohol and aldehyde end groups are determined by spectroscopic methods (*5*—*9*), wet chemical methods (*10*, *11*), and pyrolysis mass spectrometry (*12*). However, these procedures, except the last, often require a large amount of

sample (greater than milligram scale), multistep sample preparation prior to analyses, and intricate techniques, with a long analysis time. Therefore, there is considerable interest in developing convenient methods with high sensitivity for the analysis of cinnamyl alcohol and aldehyde end groups in lignins. In particular, recent studies on cinnamyl alcohol dehydrogenase deficient plants have enhanced interest in the analysis of both end groups from the viewpoint of biosynthetic and industrial concerns (*12*—*20*).

A pyrolysis method involving methylation with tetramethyl-ammonium hydroxide (TMAH) has emerged as a powerful method for characterizing polymers as shown by Challinor (*21*). Recent studies (*22*—*25*) clarify that this method is a thermally assisted chemolytic reaction, so-called TMAH thermochemolysis. This method provides more structural information than conventional pyrolysis because it renders polar products released from the polymers by thermally assisted hydrolysis, amenable to chromatographic analysis by subsequent methylation. Nowadays it is widely used to analyze lignins (*26*—*33*). Furthermore, this method is also used to characterize the chemical composition of degraded woods (*28*, *34*—*36*) and agricultural wastes (*37*, *38*) and to track changes in lignin composition during commercial growth of button, shiitake, and oyster mushroom on

* Corresponding author (telephone/fax +81-92 642 2988; e-mail kenfumi@agr.kyushu-u.ac.jp).
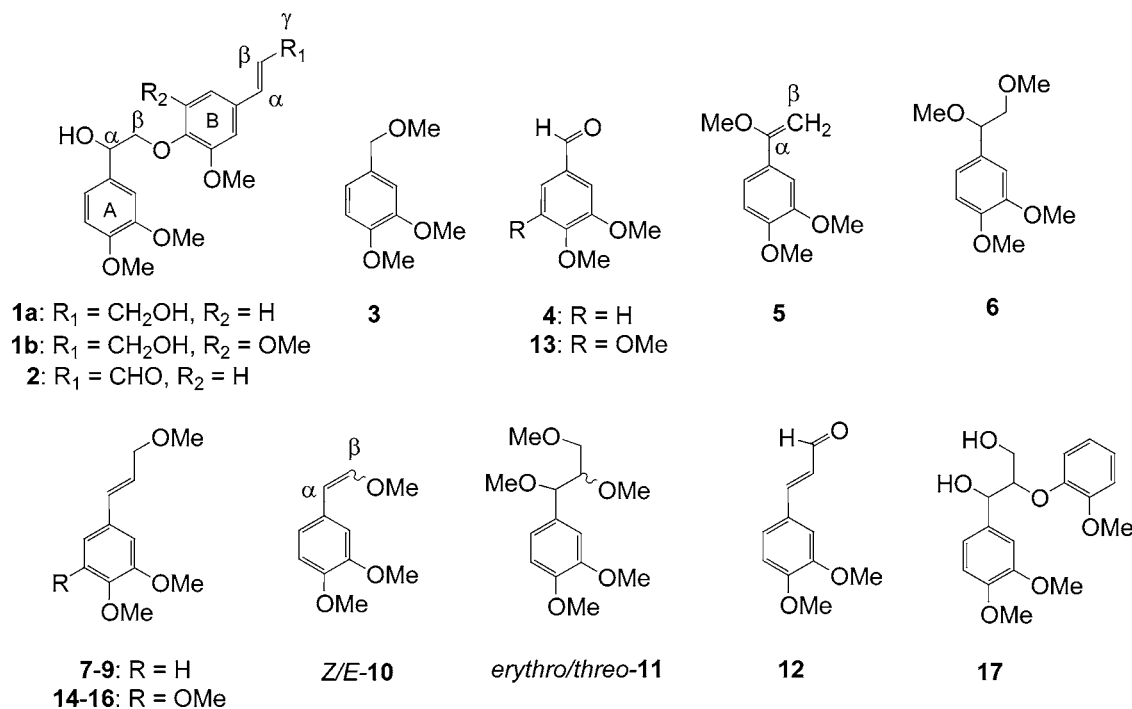† Kyushu University.
‡ University of Tsukuba.

Figure 1. Chemical structures for **1**–**17**.

woody substrates, namely, wheat straw and oak wood (*38–40*), as well as fruit-bearing trees (*41*). Despite extensive applications to the lignin analysis, however, how TMAH thermochemolysis works on lignin is still far from completely understood. Unfortunately, deciphering the lignin structure is presently difficult from the TMAH thermochemolysis product data. To use this method effectively as a tool for analyzing lignin polymer, therefore, a comprehensive understanding of TMAH thermochemolysis is required.

We are presently exploring the feasibility of TMAH thermochemolysis for analyzing lignin (*29–31, 33, 42, 43*). Recent studies showed that TMAH thermochemolysis provides β-aryl ether (*24, 33*), β-5 (*33, 43*), and β-β (*33, 42*) substructure-derived products from lignin. Along with these products, coniferyl alcohol dimethyl ether is produced from guaiacyl lignins (*33, 42–45*), suggesting that this method makes the analysis of cinnamyl alcohol and aldehyde end groups possible. If so, it could become a powerful tool for analyzing cinnamyl end groups. However, available literature references contain few reports on the TMAH thermochemolysis behavior of the end groups.

The goal of this study was to clarify the TMAH thermochemolytic behavior of the end groups in lignin. For this, dimeric lignin model compounds **1a**, **1b**, and **2** (containing a 4-*O*-etherified coniferyl alcohol, sinapyl alcohol, and coniferyl aldehyde at the β-position, respectively) were used. The results of the model compounds were compared with those of TMAH thermochemolysis of guaiacyl synthetic and native lignins.

MATERIALS AND METHODS

Melting points were uncorrected. Column chromatography was carried out on Wakogel C200 SiO_2 (Wako Pure Chemical Industries, Osaka, Japan). Thin-layer chromatography (TLC) was performed on Kieselgel 60 F_{254} silica gel 20 μm thickness on aluminum sheet (Merck). Spots were made visible with UV light. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ with a Bruker Avance-500 spectrometer and reported by chemical shifts (relative to tetramethyl-silane), splitting patterns, integration areas, and proton assignments.

Mass spectrometry (MS) analyses employed the same conditions as those employed previously (*43*).

Materials. The following materials were commercial products (Aldrich, Milwaukee, WI): coniferyl alcohol, coniferyl aldehyde, sinapyl alcohol, sinapyl aldehyde, and a 25% TMAH methanolic solution.

A bulk dehydrogenation polymer of coniferyl alcohol and a Japanese cedar (*Cryptomeria japonica*) wood used were the same as those used previously (*43*). A bulk dehydrogenation polymer of coniferyl aldehyde was prepared according to a method similar to that used in the preparation of coniferyl alcohol-DHP: yield, 19%.

β-(Coniferyl alcohol) ether of veratrylglycol, 1-(3,4-dimethoxyphe-nyl)-2-[4-(3-hydroxypropenyl)-2-methoxyphenoxy]ethanol (**1a**), was prepared according to the method of Lu and Ralph (*10*). Structure confirmation was provided by NMR and MS analyses; the numbering system of the side chain and the ring is given in **Figure 1**. Coniferyl alcohol (350 mg, 1.94 mmol) and β-bromoacetoveratrone [mp 80 °C, lit. 80−81 °C (*46*), 350 mg, 1.36 mmol] were dissolved in acetone (50 mL), and the mixture was heated under reflux over anhydrous K_2CO_3 (800 mg) with stirring for 30 min. After the mixture had been cooled, the inorganic materials were filtered off and washed with EtOAc. The combined filtrate and washings were partitioned between EtOAc and brine. After drying over Na_2SO_4 overnight, the ethyl acetate was concentrated in vacuo at <40 °C. The residue was chromatographed on 19 g of SiO_2 with a mixture of CH_2Cl_2 and EtOAc (1:1, v/v) to provide an oily ketone (460 mg, 1.34 mmol), which was crystallized from MeOH: mp 149−150 °C (MeOH); direct MS, *m/z* (%) 358 (M$^+$, 12), 165 (100), 151 (14). To the ketone (150 mg, 0.42 mmol) in a mixture of dioxane (10 mL) and H_2O (3 mL) was added $NaBH_4$ (30 mg). After 8 h of continuous stirring, the reaction was quenched by the addition of a small amount of acetic acid. The reaction mixture was partitioned in EtOAc and brine. The EtOAc was dried over Na_2SO_4 (overnight) and concentrated in vacuo at <40 °C to provide an oil (120 mg, 0.33 mmol) with purity by TLC (CH_2Cl_2/EtOH 100:3, v/v): ^{1}H NMR (acetate) δ 2.10 (6H, s, 2 × OCOCH$_3$), 3.85 (3H, s, OCH$_3$), 3.87 (3H, s, OCH$_3$), 3.91 (3H, s, OCH$_3$), 4.20 (1H, dd, *J* = 11.0, 3.9 Hz, Aβ1), 4.31 (1H, dd, *J* = 11.0, 8.4 Hz, Aβ2), 4.71 (2H, dd, *J* = 6.6, 1.1 Hz, Bγ), 6.10 (1H, dd, *J* = 8.0, 3.9 Hz, Aα), 6.17 (1H, dt, *J* = 15.8, 6.6 Hz, Bβ), 6.57 (1H, d, *J* = 15.9 Hz, Bα), 6.84−6.98 (6H, m, aromatic-H); MS (TMS), *m/z* (%) 504 (M$^+$, 4), 324 (10), 253 (17), 239 (100), 73 (57).

TMAH Thermochemolysis of Lignin

J. Agric. Food Chem., Vol. 53, No. 23, 2005   **8861**

β-(Sinapyl alcohol) ether of veratrylglycol, 1-(3,4-dimethoxyphenyl)-2-[4-(3-hydroxypropenyl)-2,6-dimethoxyphenoxy]ethanol (**1b**), was prepared in the same way as **1a** by the NaBH$_4$ reduction of 2-[4-(3-hydroxyprop-1-enyl)-2,6-dimethoxyphenoxy]-1-(4-hydroxy-3-methoxyphenyl)ethan-1-one: oil with purity by TLC (CH$_2$Cl$_2$/EtOH 100:3, v/v): ^{1}H NMR (acetate) δ 2.09 (3H, s, OCOCH$_3$), 2.12 (3H, s, OCOCH$_3$), 3.82 (6H, s, OCH$_3$), 3.86 (3H, s, OCH$_3$), 3.88 (3H, s, OCH$_3$), 4.20 (1H, dd, *J* = 11.0, 3.9 Hz, Aβ1), 4.31 (1H, dd, *J* = 11.0, 8.4 Hz, Aβ2), 4.71 (2H, dd, *J* = 6.6, 1.1 Hz, Bγ), 6.01 (1H, dd, *J* = 8.8, 3.3 Hz, Aα), 6.21 (1H, dt, *J* = 15.8, 6.5 Hz, Bβ), 6.58 (1H, d, *J* = 15.9 Hz, Bα), 6.62 (2H, s, aromatic-H), 6.83 (1H, d, *J* = 8.3 Hz, aromatic-H), 6.90 (1H, d, *J* = 2.0 Hz, aromatic-H), 6.93 (1H, dd, *J* = 8.3, 2.0 Hz, aromatic-H); MS (TMS), *m/z* (%) 534 (M$^+$, 2), 239 (100), 73 (60).

β-(Coniferyl aldehyde) ether of veratrylglycol, 3-{4-[2-(3,4-dimethoxyphenyl)-2-hydroxyethoxy]-3-methoxyphenyl}prop-2-enal (**2**), was prepared by heating a mixture of **1a** (1.07 g) and 1,4-benzoquinone (0.62 g) in 7 mL of diglyme at 120 °C for 24 h under stirring according to the method of Kulkarni and Sebastian (*47*). The reaction mixture was poured into water, followed by extraction with EtOAc. After being washed with brine, the EtOAc was dried over Na$_2$SO$_4$ overnight and concentrated in vacuo at <40 °C. The residue was chromatographed on SiO$_2$ with hexane/EtOAc (1.5:1, v/v) to provide yellowish **2**, which was crystallized from MeOH in a refrigerator (0.44 g): mp 147−148 °C (MeOH × 2); ^{1}H NMR (acetate) δ 2.10 (3H, s, OCOCH$_3$), 3.88 (3H, s, OCH$_3$), 3.89 (3H, s, OCH$_3$), 3.91 (3H, s, OCH$_3$), 4.26 (1H, dd, *J* = 10.8, 3.9 Hz, Aβ1), 4.36 (1H, dd, *J* = 10.8, 8.0 Hz, Aβ2), 6.13 (1H, dd, *J* = 7.8, 3.9 Hz, Aα), 6.61 (1H, dd, *J* = 15.8, 7.7 Hz, Bβ), 6.87 (1H, d, *J* = 8.7 Hz, aromatic-H), 6.92 (1H, d, *J* = 8.3 Hz, aromatic-H), 6.98 (1H, s, aromatic-H), 6.99 (1H, d, *J* = 1.8 Hz, aromatic-H), 7.09 (1H, d, *J* = 1.8 Hz, aromatic-H), 7.13 (1H, dd, *J* = 8.4, 1.8 Hz, aromatic-H), 7.40 (1H, d, *J* = 15.8 Hz, Bα), 9.65 (1H, d, *J* = 7.7 Hz, Bγ); MS (TMS ether), *m/z* (%) 430 (M$^+$, <1), 340 (2), 281 (15), 240 (19), 239 (100), 207 (39), 73 (30).

TMAH Thermochemolysis−Gas Chromatography−Mass Spectrometry (GC-MS). The TMAH thermochemolysis-GC-MS system was a combination of a JHP-3 model Curie-point pyrolyzer (Japan Analytical Industry, Tokyo, Japan) and an HP 5890 series II gas chromatograph (Hewlett-Packard, Palo Alto, CA) with an HP 5972A quadrupole mass selective detector (Hewlett-Packard). Samples were placed on a 50 μm ferromagnetic pyrofoil. The 25% TMAH methanolic solution (∼3−5 μL) was added to the model compounds (∼20 μg) and coniferyl aldehyde-DHP (∼50−70 μg) on the pyrofoil with a syringe. After the mixture had been left for ∼3 min at room temperature to remove most of the MeOH, the syrupy mixture was tightly wrapped in the pyrofoil to ensure contact between the mixture and the pyrofoil. The sample-loaded pyrofoil was inserted into a sample tube. After the pyrolysis system had been flushed with helium gas for 15 s, the sample holder with the sample tube was centered in the pyrolyzer heated at 250 °C. The samples were pyrolyzed at 315 °C for 4 s under a flow of helium carrier gas. TMAH thermochemolysis of the coniferyl alcohol-DHP and the cedar wood was the same as that described previously (*43*). The volatile products were sent to the GC-MS and subjected to the MS analyses. The products were identified by comparing the retention times of standard compounds or their on-line TMAH/methylation products with those of the products and the MS data of the products with published data (*48*).

TMAH Thermochemolysis-GC. The pyrolysis-GC system was a combination of a JHP-3 model Curie-point pyrolyzer (Japan Analytical Industry) and a Shimadzu GC-17A gas chromatograph (Shimadzu, Kyoto, Japan) with a flame ionization detector (FID) and a 1:50 split ratio injector. The TMAH thermochemolysis-GC runs were performed similarly to the TMAH thermochemolysis-GC-MS runs. Product identifications were carried out on the basis of the TMAH thermochemolysis-GC-MS results.

On-Line Methylation of Standard Materials with TMAH. The 25% TMAH methanolic solution containing a standard material (coniferyl alcohol, coniferyl aldehyde, sinapyl alcohol, and sinapyl aldehyde) was introduced into the GC-MS or GC injection port heated at 280 °C and the product analysis was done according to the TMAH thermochemolysis-GC-MS results.
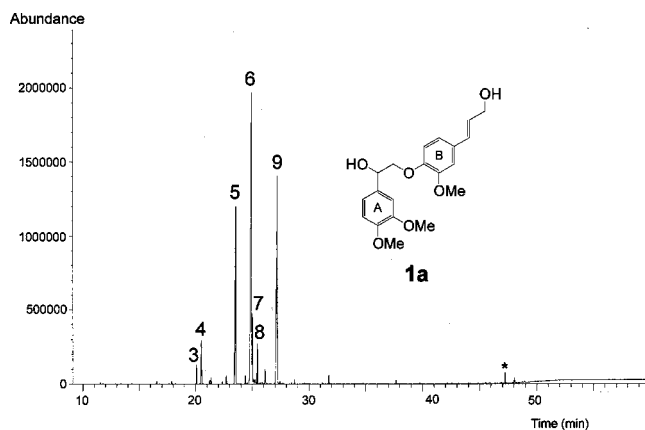

Figure 2. TMAH thermochemolysis-GC-MS trace of **1a**. TMAH thermochemolysis was performed at 315 °C for 4 s. Product names and structures refer to those in **Table 1** and **Figure 1**. Methylated **1a**, 3-{4-[2-(3,4-dimethoxyphenyl)-2-methoxyethoxy]-3-methoxyphenyl}-1-methoxyprop-2-ene, is labeled with an asterisk: MS *m/z* (%) 388 (2), 181 (100).

Table 1. Identified TMAH Thermochemolysis Products of **1a**

peak	product	MS data, *m/z* (rel intensity %)	rel mol %^{a}	source
3	veratryl alcohol methyl ether	182 (M$^+$, 48), 151 (100)	2.6	A moiety
4	veratryl aldehyde	166 (M$^+$, 100), 165 (64)	5.8	B
5	α,3,4-trimethoxystyrene^b	194 (M$^+$, 100), 179 (20), 163 (44), 151 (34)	16.3	A
6	veratrylglycol dimethyl ether^c	226 (M$^+$, 3), 181 (100), 166 (22)	41.9	A
7	isomer of **9**	208 (M$^+$, 100), 177 (84), 145 (23)	4.4	B
8	isomer of **9**	208 (M$^+$, 100), 177 (89), 145 (24)	3.9	B
9	coniferyl alcohol dimethyl ether	208 (M$^+$, 88), 177 (100), 146 (37)	25.1	B
	total		100	

^{a} Calculated on the basis of effective carbon numbers of the products and their GC signal areas between 10 and 40 min of retention time. ^{b} 1-(3,4-Dimethoxyphenyl)-1-methoxyethene. ^{c} 4-(1,2-Dimethoxyethyl)-1,2-dimethoxybenzene.

RESULTS AND DISCUSSION

TMAH Thermochemolysis of 1a. Figure 2 shows the total ion chromatogram (TIC) of TMAH thermochemolysis (315 °C for 4 s) of **1a**. **Table 1** lists the identified pyrolysis products with MS data, relative abundances (mole percent), and pyrolytic sources. The relative product yields were determined using the effective carbon numbers and GC-FID signal areas of the products (*29*, *49*). The TIC reveals a large abundance of monomeric products between retention times of 20 and 30 min and a small abundance of dimeric products including the permethylated product of **1a** (marked with an asterisk), 3-{4-[2-(3,4-dimethoxyphenyl)-2-methoxyethoxy]-3-methoxyphenyl}-1-methoxyprop-2-ene [MS *m/z* (%) 388 (2), 181 (100)]. This shows that the β-O-4 linkage of **1a** was effectively cleaved during the 315 °C/4 s TMAH thermochemolysis.

TMAH thermochemolysis cleaves the β-O-4 linkage of **1a** followed by permethylation of the released coniferyl alcohol, resulting in the formation of coniferyl alcohol dimethyl ether (MW 208). **Figure 2** reveals three products **7−9** having the molecular ion at *m/z* 208 in a 14:10:100 GC-MS signal area ratio (order of GC-MS elution time). These are isomeric because of the similarity of their mass fragmentation patterns. The difference in mass fragmentation patterns was that **7** and **8** have a base peak at *m/z* 208, and **9** has it at *m/z* 177. Although coniferyl alcohol dimethyl ether should have only two isomers (*Z/E* form with the side-chain double bond), treatment of **1a** with TMAH provides the third isomer as shown in **Figure 2**,
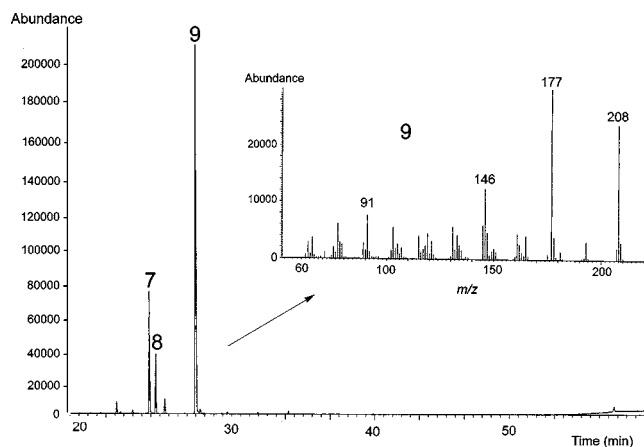
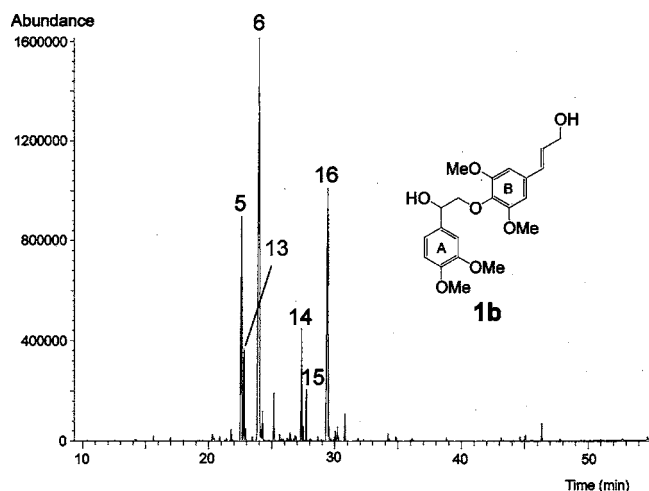**Figure 3.** On-line TMAH methylation GC-MS trace of coniferyl alcohol.

Figure 4. TMAH thermochemolysis-GC-MS trace of **1b**. TMAH thermochemolysis was performed at 315 °C for 4 s. Product structures refer to those in **Figure 1**.

which is unknown at present. On-line methylation of coniferyl alcohol with TMAH also provided **7**−**9** in a similar GC-MS signal area ratio (12:4:100 in order of GC-MS elution time) (**Figure 3**). The isomeric ratio may depend on the pyrolysis temperature employed because TMAH thermochemolysis at high temperature enhanced the contribution of **7** and **8**; for example, TMAH thermochemolysis of coniferyl alcohol at 500 °C for 4 s provided **7**, **8**, and **9** in a 60:40:100 GC-MS signal area ratio (*48*).

Similarly, **1b** (**Figure 4**) provided sinapyl alcohol dimethyl ether consisting of three isomers, **14**−**16**, in a 25:9:100 GC-MS signal area ratio (order of GC-MS elution time) in TMAH thermochemolysis, and sinapyl alcohol provided **14**−**16** also by both TMAH thermochemolysis and on-line methylation with TMAH.

Cleavage of the *β-O*-4 linkage of **1a** also resulted in veratrylglycol, 1-(3,4-dimethoxyphenyl)ethane-1,2-diol, as a counterpart of coniferyl alcohol released from the B moiety. The glycol was immediately permethylated to provide **6** in ∼42 mol % yield. However, α-methoxystyrene **5**, a subproduct, was also produced in ∼16 mol % yield. The formation of **5** is due to dehydration between the Cα-proton and the Cβ-OH group of the released veratrylglycol, followed by methylation of the Cα-OH. On the other hand, lignin produces *β*-methoxystyrenes consisting of two isomers such as *Z/E*-**10** (*29*, *31*, *33*, *43*), due to the combination of (1) dehydration between the α-OH group and the *β*-proton of the phenylglycerol moieties released by split

of *β-O*-4 linkages, (2) simultaneous elimination of the *γ*-CH₂-OH group, and (3) permethylation. Although **5** and **10** have the same molecular ion at *m/z* 194 (rel int 100%), **10** is distinguishable from **5** by a large abundance of the *m/z* 179 ion (65%) and the small abundance of the *m/z* 163 ion (1%) present in the MS spectrum of **10** (*48*). The formation of α-methoxy-styrenes may be evidence for the presence of the phenylglycol moieties involved in *β-O*-4 linkages.

The yield of **6** is roughly 2.5 times that of **5**, suggesting that in TMAH thermochemolysis of nonphenolic *β*-aryl ether substructures the methylation is favored over the dehydration. In fact, TMAH thermochemolysis of permethylated woods with diazomethane provided phenyltrimethoxypropanes such as *erythro/threo*-**11** in a larger abundance than *β*-methoxystyrenes such as *Z/E*-**10**, in contrast to the nonmethylated woods (*31*).

The contributions of **5**+**6** and **7**+**8**+**9** are ∼58 and ∼33 relative mol %, respectively. The low contribution of coniferyl alcohol dimethyl ether (**5**+**6**/**7**+**8**+**9** ratio ∼1.7:1) demonstrates the low derivatization efficiency of the coniferyl alcohol released during TMAH thermochemolysis to coniferyl alcohol dimethyl ether. The nonmethylated coniferyl alcohol may polymerize.

Pyrolysis in the absence of TMAH produces coniferyl alcohol from both coniferyl alcohol end groups and guaiacylglycerol moieties involved in *β-O*-4 linkages (*50*). However, the latter is not responsible for the formation of **7**−**9** because the TMAH thermochemolysis pyrolysate of veratrylglycerol-*β*-guaiacyl ether (**17**) is devoid of **7**−**9** (*30*, *31*). From the findings on the TMAH thermochemolysis behavior of **1** and **17**, therefore, it is clear that coniferyl alcohol dimethyl ether mostly comes from coniferyl alcohol end groups.

The source of **4** is the coniferyl alcohol moiety because the TIC of **1b** (**Figure 4**) reveals 3,4,5-trimethoxybenzaldehyde (**13**) and is devoid of **4**, which should stem from the A moiety. However, the coniferyl alcohol moiety makes a small contribution to the formation of **4**. In TMAH thermochemolysis studies on microbial decay of lignin in native, managed, and cultured systems (*28*, *34*, *36*, *40*, *41*), the yield ratios of benzoic acid methyl esters such as 3,4-dimethoxybenzoic acid methyl ester to benzaldehydes such as **4** (Ad/Al), the larger values of which indicate increased oxidation state of the lignin, are used widely as an indicator to imply the extent of the structural change of the lignin. Our finding on the formation of **4** therefore shows that use of the Ad/Al values evaluated in TMAH thermochemolysis of lignin should be treated with caution because the additional Al contribution could lead to the misinterpretation that a compost or woody substrate has undergone less degradation than is the case.

TMAH Thermochemolysis of 2. Figure 5a shows the TMAH thermochemolysis-GC-MS trace of **2**. No methylated **2** was revealed, showing that the *β-O*-4 linkage was effectively cleaved, as in TMAH thermochemolysis of **1a** and **1b**. The counterparts of the coniferyl aldehyde released by the *β*-ether linkage cleavage, **5** and **6**, were also observed. However, we observed no expected coniferyl aldehyde methyl ether (**12**) [MS (*m/z*) 192 (M⁺, 100), 161 (87)], which appears at a 27.5 min retention time. Instead, **4** was obtained in an appreciable abundance. Its contribution (**4**:**5**:**6** molar ratio of ∼28:22:50) increased, compared to that (∼6:16:42) in **Figure 1**. Aldehyde **4** may come from the released coniferyl aldehyde.

To confirm this, coniferyl aldehyde itself and its bulk DHP were subjected to the 315 °C/4 s TMAH thermochemolysis. Coniferyl aldehyde provided **12** in a trace abundance and **4** as the major product (the pyrogram is not shown), like **2**. Similar results were obtained with both TMAH thermochemolysis and
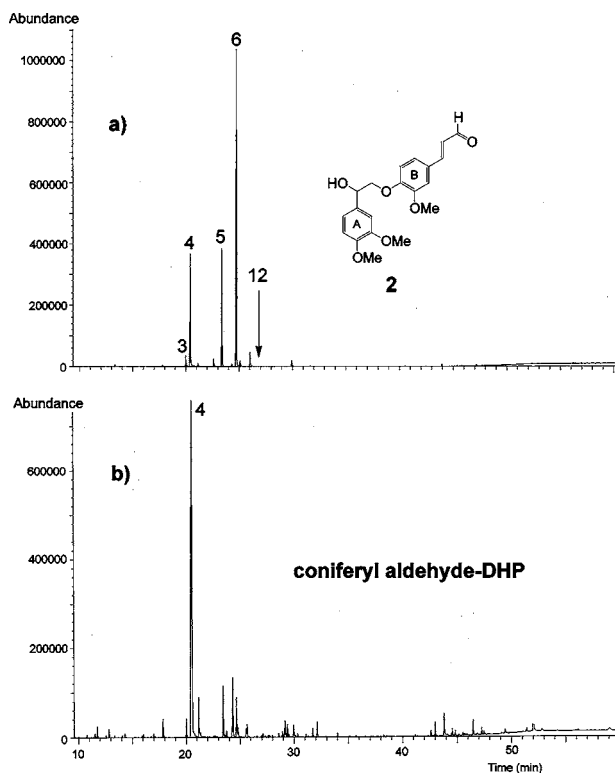
TMAH Thermochemolysis of Lignin

J. Agric. Food Chem., Vol. 53, No. 23, 2005   **8863**

Figure 5. TMAH thermochemolysis-GC-MS traces of (**a**) **2** and (**b**) coniferyl aldehyde-DHP. TMAH thermochemolysis was performed at 315 °C for 4 s. Product names and structures refer to those in **Table 1** and **Figure 1**.
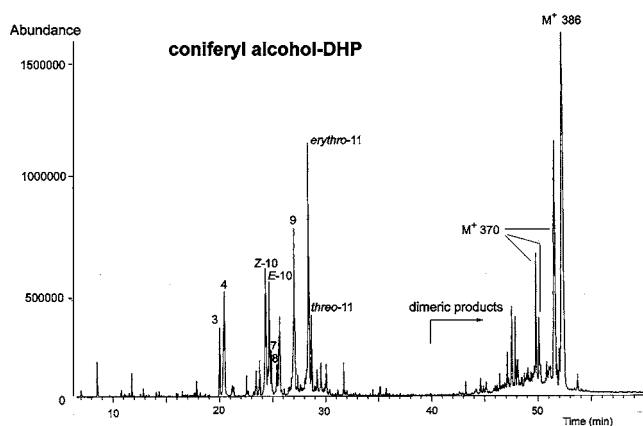

Figure 6. TMAH thermochemolysis-GC-MS trace of coniferyl alcohol-DHP. TMAH thermochemolysis was performed at 500 °C for 4 s (*43*). Product names and structures refer to those in **Table 1** and **Figure 1**.
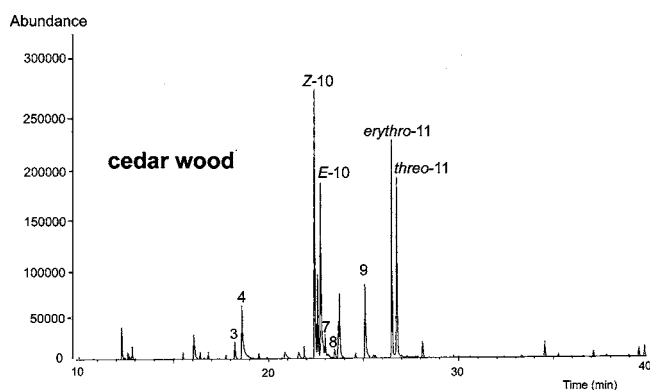

Figure 7. TMAH thermochemolysis-GC-MS trace of a Japanese cedar wood. TMAH thermochemolysis was performed at 500 °C for 4 s (*43*). Product names and structures refer to those in **Table 1** and **Figure 1**.

on-line TMAH methylation of sinapyl aldehyde, which provided 3,4,5-trimethoxybenzaldehyde as the major product, and a trace abundance of sinapyl aldehyde methyl ether. Coniferyl aldehyde-DHP results in the pyrogram (**Figure 5b**) with a large abundance of **4** and a scarcity of **12**. Therefore, in TMAH thermochemolysis of **2** the large participation of 4-*O*-etherified coniferyl aldehyde is clear in the formation of **4**.

Although Filley et al. (*24*) demonstrate that guaiacylglycerol moieties involved in β-aryl ether linkages are responsible for the formation of **4**, we here propose 4-*O*-etherified coniferyl aldehyde as a new source for **4** in TMAH thermochemolysis of lignin. The formation of **4** from **1** having no coniferyl aldehyde group shows that during TMAH thermochemolysis parts of 4-*O*-etherified coniferyl alcohol are oxidized to 4-*O*-etherified coniferyl aldehyde to produce **4**. These findings demonstrate that TMAH thermochemolysis provides little information on cinnamyl aldehyde end groups from lignin.

Coniferyl Alcohol Dimethyl Ether Isomers 7−9 in the TMAH Thermochemolysis Pyrolysates of Guaiacyl Synthetic and Native Lignins. Figure 6 shows the TMAH thermochemolysis-GC-MS trace of coniferyl alcohol-DHP. Clearly, **7−9** are produced. In particular, **9** is revealed as a distinct, intense signal, following *erythro*-**11**. Products **4** and *Z/E*-**10** are observed in a smaller abundance than **9**. On the basis of the GC-MS signal areas the summed contribution of **7−9** is ∼0.40 that of the guaiacylglycerol moiety-derived products (**10+11**). The M⁺ 370 isomers and the M⁺ 386 product revealed above a 40 min retention time are derived from dehydrodiconiferyl alcohol (*43*) and pinoresinol (*42*) type substructures, respectively. Parts of **4** stem from 4-*O*-linked coniferyl alcohol in the DHP, as described above.

The cedar wood provides a different product profile (**Figure 7**) from the coniferyl alcohol-DHP. The pyrogram is predominated by *Z/E*-**10** and *erythro/threo*-**11**. Isomers **7−9** were

consequently revealed in a smaller abundance than in **Figure 6**. Although they were revealed in GC-MS determinable abundances, their peak intensities were very weak. The summed contribution of **7−9** was ∼0.15 of the **10+11** contribution, based on their GC-MS signal areas. The difference in abundance of **7−9** observed between the two TICs reflects the difference in abundance of coniferyl alcohol end groups in the lignins because DHPs and milled wood lignins contain the end groups in large and small abundances, respectively: 10% in a DHP (*51*) and 3% in spruce milled wood lignin (*2*).

Although **4** was observed in a small abundance in the TIC of the cedar wood, **12** was not observed despite the fact that ∼3−5.3% of 4-*O*-etherified coniferyl aldehyde in a spruce milled wood lignin is reported (*3−5, 8, 9*). This may be responsible for the limitation of the TMAH thermochemolysis application to the cinnamyl aldehyde end groups analysis as described above or the very small frequency of 4-*O*-etherified coniferyl aldehyde present in the cedar wood. Pyrolysis in the presence of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide suggested the latter possibility (*52*).

In conclusion, cinnamyl alcohol end groups in lignin provide the corresponding permethylated alcohols consisting of three isomers in a GC-MS detectable amount in TMAH thermochemolysis. In particular, the isomer with the slowest retention time, such as **9**, is characteristic of 4-*O*-etherified cinnamyl alcohols because it is revealed as an intense GC-MS signal distinguishable from other TMAH thermochemolysis products. Therefore, TMAH thermochemolysis is capable of drawing information on cinnamyl alcohol end groups in lignin. However,
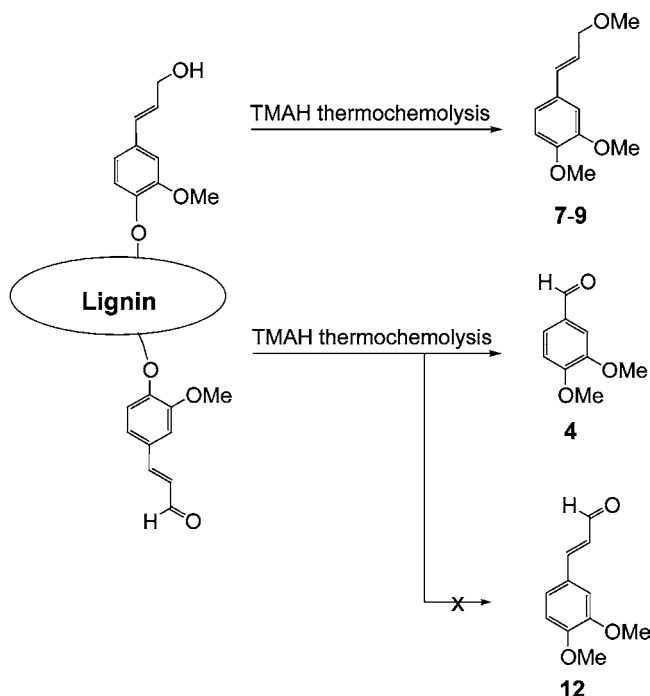
Figure 8. Behavior of 4-*O*-etherified coniferyl alcohol and aldehyde in lignin in TMAH thermochemolysis.

it is not informative on cinnamyl aldehyde end groups because it fails to provide cinnamyl aldehyde methyl ethers. The results obtained are given in **Figure 8**, in which the behavior of cinnamyl end groups in TMAH thermochemolysis is briefly demonstrated as exemplified by softwood lignin.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Sarkanen, K. V.; Ludwig, C. H. Definition and nomenclature. In *Lignins: Occurrence, Formation, Structure and Reactions*; Sarkanen, K. V., Ludwig, C., Eds.; Wiley: New York, 1971; pp 1−18.

(2) Goldschmid, O. Ultraviolet spectra. In *Lignins: Occurrence, Formation, Structure and Reactions*; Sarkanen, K. V., Ludwig, C., Eds.; Wiley: New York, 1971; pp 241−266.

(3) Adler, E. Lignin chemistry−past, present and future. *J. Wood Sci. Technol.* **1977**, *11*, 169−218.

(4) Lai, Y.-Z.; Sarkanen, K. V. Isolation and structural studies. In *Lignins: Occurrence, Formation, Structure and Reactions*; Sarkanen, K. V., Ludwig, C. H., Eds.; Wiley: New York, 1971; pp 165−240.

(5) Marton, J.; Adler, E. Carbonyl groups in lignin. III. Mild catalytic hydrogenation of Björkman lignin. *Acta Chem. Scand.* **1961**, *15*, 370−387.

(6) Lindgren, B. O.; Mikawa, H. The presence of cinnamyl alcohol groups in lignin. *Acta Chem. Scand.* **1957**, *11*, 826−835.

(7) Terashima, N.; Atalla, R. H.; Vanderhart, D. L. Solid state NMR spectroscopy of specifically ^{13}C-enriched lignin in wheat straw from coniferin. *Phytochemistry* **1997**, *46*, 863−870.

(8) Zhang, L.; Gellerstedt, G. Detection and determination of carbonyls and quinones by modern NMR techniques. *Proceedings of the 10th International Symposium on Wood and Pulping Chemistry*; Japan Tappi: Yokohama, Japan, 1999; Vol. II, pp 164−170.

(9) Lundquist, K.; Olsson, T. NMR studies of lignins. 1. Signals due to protons in formyl groups. *Acta Chem. Scand.* **1977**, *B31*, 788−792.

(10) Lu, F.; Ralph, J. The DFRC method for lignin analysis. 7. Behavior of cinnamyl end groups. *J. Agric. Food Chem.* **1999**, *47*, 1981−1987.

(11) Rolando, C.; Monties, B.; Lapierre, C. Thioacidolysis. In *Methods in Lignin Chemistry*; Lin, S. Y., Dence, C. W., Eds.; Springer-Verlag: Berlin, Germany, 1992; pp 334−349.

(12) MacKay, J.; Dimmel, D. R.; Boon, J. J. Pyrolysis mass spectral characterization of wood from CAD-deficient pine. *J. Wood Chem. Technol.* **2001**, *21*, 19−29.

(13) Ralph, J.; MacKay, J. J.; Hatfield, R. D.; Omalley, D. M.; Whetten, R. W.; Sederoff, R. R. Abnormal lignin in a loblolly pine mutant. *Science* **1997**, *277*, 235−239.

(14) Kim, H.; Ralph, J.; Yahiaoui, N.; Pean, M.; Boudet, A. M. Cross-coupling of hydroxycinnamyl aldehydes into lignins. *Org. Lett.* **2000**, *2*, 2197−2200.

(15) Dimmel, D. R.; MacKay, J. J.; Althen, E. M.; Parks, C.; Sederoff, R. R. Pulping and bleaching of CAD-deficient wood. *J. Wood Chem. Technol.* **2001**, *21*, 1−17.

(16) Ito, T.; Hayase, R.; Kawai, S.; Ohashi, H.; Higuchi, T. Coniferyl aldehyde dimers in dehydrogenative polymerization: model of abnormal lignin formation in cinnamyl alcohol dehydrogenase-deficient plants. *J. Wood Sci.* **2002**, *48*, 216−221.

(17) Pomar, F.; Merino, F.; Barcelo, A. R. *O*-4-Linked coniferyl and sinapyl aldehydes in lignifying cell walls are the main targets of the Wiesner (phloroglucinol-HCl) reaction. *Protoplasma* **2002**, *220*, 17−38.

(18) Li, L.; Zhou, Y. H.; Cheng, X. F.; Sun, J. Y.; Marita, J. M.; Ralph, J.; Chiang, V. L. Combinatorial modification of multiple lignin traits in trees through multigene cotransformation. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4939−4944.

(19) Kim, H.; Ralph, J.; Lu, F.; Ralph, S. A.; Boudet, A.-M.; MacKay, J. J.; Sederoff, R. R.; Ito, T.; Kawai, S.; Ohashi, H.; Higuchi, T. NMR analysis of lignins in CAD-deficient plants. Part 1. Incorporation of hydroxycinnamaldehydes and hydroxybenzaldehydes into lignins. *Org. Biomol. Chem.* **2003**, *1*, 268−281.

(20) Barriere, M.; Ralph, J.; Mechin, V.; Guillaumie, S.; Grabber, J.; Argillier, O.; Chabbert, B.; Lapierre, C. Genetic and molecular basis of grass cell wall biosynthesis and degradability. II. Lessons from brown-midrib mutants. *C. R. Biol.* **2004**, *327*, 847−860.

(21) Challinor, J. M. A pyrolysis-derivatisation-gas chromatography technique for the structural elucidation of some synthetic polymers. *J. Anal. Appl. Pyrolysis* **1989**, *16*, 323−333.

(22) Challinor, J. M. Characterisation of wood by pyrolysis derivatisation-gas chromatography/mass spectrometry. *J. Anal. Appl. Pyrolysis* **1995**, *35*, 93−107.

(23) Clifford, D. J.; Carson, D. M.; McKinney, D. E.; Bortiatynski, J. M.; Hatcher, P. G. A new rapid technique for the characterization of lignin in vascular plants: thermochemolysis with tetramethylammonium hydroxide (TMAH). *Org. Geochem.* **1995**, *23*, 169−175.

(24) Filley, T. R.; Minard, R. D.; Hatcher, P. G. Tetramethylammonium hydroxide (TMAH) thermochemolysis: proposed mechanisms based upon the application of ^{13}C-labeled TMAH to a synthetic model lignin dimer. *Org. Geochem.* **1999**, *30*, 607−621.

(25) Challinor, J. M. Review: The development and applications of thermally assisted hydrolysis and methylation reactions. *J. Anal. Appl. Pyrolysis* **2001**, *61*, 3−34.

(26) Hatcher, P. G.; Minard, R. D. Comparison of dehydrogenase polymer (DHP) lignin with native lignin from gymnosperm wood by thermochemolysis using tetramethylammonium hydroxide (TMAH). *Org. Geochem.* **1996**, *24*, 593−600.

(27) Hardell, H.-L.; Nilvebrant, N.-O. Analytical pyrolysis of spruce milled wood lignins in the presence of tetramethylammonium hydroxide. *Nord. Pulp Pap. Res. J.* **1996**, *2*, 121−126.

(28) del Rio, J. C.; McKinney, D. E.; Knicker, H.; Nanny, M. A.; Minard, R. D.; Hatcher, P. G. Structural characterization of bio- and geo-macromolecules by off-line thermochemolysis with tetramethylammonium hydroxide. *J. Chromatogr. A* **1998**, *823*, 433−448.

TMAH Thermochemolysis of Lignin

J. Agric. Food Chem., Vol. 53, No. 23, 2005 **8865**

(29) Kuroda, K.; Ozawa, T.; Ueno, T. Characterization of sago palm (*Metroxylon sagu*) lignin by analytical pyrolysis. *J. Agric. Food Chem.* **2001**, *49*, 1840−1847.

(30) Kuroda, K.; Izumi, A.; Mazumder, B. B.; Ohtani, Y.; Sameshima, K. Characterization of kenaf (*Hibiscus cannabinus*) lignin by pyrolysis−gas chromatography−mass spectrometry in the presence of tetramethylammonium hydroxide. *J. Anal. Appl. Pyrolysis* **2002**, *64*, 453−463.

(31) Kuroda, K.; Nishimura, N.; Nakagawa, A.; Dimmel, D. R. Pyrolysis of lignin in the presence of tetramethylammonium hydroxide (TMAH): a convenient method for S/G ratio determination. *J. Agric. Food Chem.* **2002**, *50*, 1022−1027.

(32) Fu, S. Y.; Lucia, L. A. TMAH-pyrolysis-gas chromatography−mass spectrometry analysis of residual lignin changes in softwood kraft pulp during oxygen delignification. *Can. J. Chem.* **2004**, *82*, 1197−1202.

(33) Kuroda, K.-I.; Nakagawa-izumi, A. Tetramethylammonium hydroxide (TMAH) thermochemolysis of guaiacyl-syringyl and guaiacyl dehydrogenation polymers. *Org. Geochem.* **2005**, *36*, 53−61.

(34) Filley, T. R.; Hatcher, P. G.; Shortle, W. C.; Praseuth, R. T. The application of ^{13}C-labeled tetramethylammonium hydroxide (^{13}C-TMAH) thermochemolysis to the study of fungal degradation of wood. *Org. Geochem.* **2000**, *31*, 181−198.

(35) Filley, T. R.; Cody, G. D.; Goodell, B.; Jellison, J.; Noser, C.; Ostrofsky, A. Lignin demethylation and polysaccharide decomposition in spruce sapwood degraded by brown rot fungi. *Org. Geochem.* **2002**, *33*, 111−124.

(36) Vane, C. H. The molecular composition of lignin in spruce decayed by white-rot fungi (*Phanerochaete chrysosporium* and *Trametes versicolor*) using pyrolysis-GC-MS and thermochemolysis with tetramethylammonium hydroxide. *Int. Biodeterior. Biodegrad.* **2003**, *51*, 67−75.

(37) Chefetz, B.; van Heemst, J. D. H.; Chen, Y.; Romaine, C. P.; Chorover, J.; Rosario, R.; Gui, M. X.; Hatcher, P. G. Organic matter transformations during the weathering process of spent mushroom substrate. *J. Environ. Qual.* **2000**, *29*, 592−602.

(38) Chen, Y.; Chefetz, B.; Rosario, R.; van Heemst, J. D. H.; Romaine, C. P.; Hatcher, P. G. Chemical nature and composition of compost during mushroom growth. *Compost Sci. Util.* **2000**, *8*, 347−359.

(39) Vane, C. H.; Martin, S. C.; Snape, C. E.; Abbott, G. D. Degradation of lignin in wheat straw during growth of the oyster mushroom (*Pleurotus ostreatus*) using off-line thermochemolysis with tetramethylammonium hydroxide and solid-state ^{13}C NMR. *J. Agric. Food Chem.* **2001**, *49*, 2709−2716.

(40) Vane, C. H.; Drage, T. C.; Snape, C. E. Biodegradation of oak (*Quercus alba*) wood during growth of the shiitake mushroom (*Lentinula edodes*): a molecular approach. *J. Agric. Food Chem.* **2003**, *51*, 947−956.

(41) Vane, C. H.; Drage, T. C.; Snape, C. E.; Stephenson, M. H.; Foster, C. Decay of cultivated apricot wood (*Prunus armeniaca*) by the ascomycete *Hypocrea sulphurea*, using solid state ^{13}C NMR and off-line TMAH thermochemolysis with GC-MS. *Int. Biodeterior. Biodegrad.* **2005**, *55*, 175−185.

(42) Nakagawa-izumi, A.; Kuroda, K.; Ozawa, T. Thermochemolytic behavior of β-β lignin structures in the presence of tetramethylammonium hydroxide (TMAH). *Org. Geochem.* **2004**, *35*, 763−774.

(43) Kuroda, K.; Nakagawa-izumi, A.; Dimmel, D. R. Pyrolysis of lignin in the presence of tetramethylammonium hydroxide (TMAH): products stemming from β-5 substructures. *J. Agric. Food Chem.* **2002**, *50*, 3396−3400.

(44) Martin, F.; del Rio, J. C.; Gonzalez-Vila, F. J.; Verdejo, T. Thermally assisted hydrolysis and alkylation of lignins in the presence of tetra-alkylammonium hydroxides. *J. Anal. Appl. Pyrolysis* **1995**, *35*, 1−13.

(45) del Rio, J. C.; Martin, F.; Gonzalez-Vila, F. J. Thermally assisted hydrolysis and alkylation as a novel pyrolytic approach for the structural characterization of natural biopolymers and geomacromolecules. *Trends Anal. Chem.* **1996**, *15*, 70−79.

(46) Erdtman, H.; Leopold, B. Aromatic keto and hydroxy polyethers as lignin models. II. *Acta Chem. Scand.* **1949**, *3*, 1358−1378.

(47) Kulkarni, M. G.; Sebastian, M. T. 1,4-Benzoquinone: a new reagent for oxidation of alcohols. *Tetrahedron Lett.* **1990**, *31*, 4497−4500.

(48) Kuroda, K. Electron-impact (EI) mass spectra of 1,2-dimethoxy-benzenes related to the pyrolysis products of guaiacyl lignin in the presence of tetramethylammonium hydroxide (TMAH). *J. Anal. Appl. Pyrolysis* **2002**, *64*, 433−451.

(49) Tong, H. Y.; Karasek, F. W. Flame ionization detector response factors for compound classes in quantitative analysis of complex organic mixtures. *Anal. Chem.* **1984**, *56*, 2124−2128.

(50) Kuroda, K. Analytical pyrolysis products derived from cinnamyl alcohol-end groups in lignins. *J. Anal. Appl. Pyrolysis* **2000**, *53*, 123−134.

(51) Brunow, G.; Lundquist, K. Comparison of a synthetic dehydrogenation polymer of coniferyl alcohol with milled wood lignin from spruce, using ^{1}H NMR spectroscopy. *Paperi ja puu* **1980**, *62*, 669−672.

(52) Nakagawa-Izumi, A.; Kuroda, K. Analysis of cinnamyl alcohol- and cinnamyl aldehyde end groups in lignin by trimethylsilylation/pyrolysis−gas chromatography. *Bunseki Kagaku* **2003**, *52*, 1159−1166.