JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Pyrolysis of Lignin in the Presence of Tetramethylammonium Hydroxide (TMAH): Products Stemming from β-5 Substructures

KEN-ICHI KURODA,*,† AKIKO NAKAGAWA-IZUMI,† AND DONALD R. DIMMEL‡

Institute of Agricultural and Forest Engineering, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan, and Institute of Paper Science and Technology, 500 10th St. N. W., Atlanta, Georgia 30318-5794

Lignin model compounds, synthetic lignins, and cedar wood have been analyzed by pyrolysis−gas chromatography(−mass spectrometry) in the presence of tetramethylammonium hydroxide (TMAH) to examine the behavior of β-5 substructures specifically under these conditions. Two model compounds contained a β-5 linkage and a γ-CH_2OH group. The phenolic model compound produced stilbene products by way of a formaldehyde elimination of the γ-CH_2OH. The nonphenolic model compound underwent dehydration to give arylbenzofuran products. Dehydrogenation polymers of coniferyl alcohol gave a large amount of stilbene products in TMAH/pyrolysis. TMAH/pyrolysis of a Japanese cedar (*Cryptomeria japonica*) wood yielded a very small amount of stilbene products. The results demonstrated that synthetic lignins are rich in terminal β-5 substructures, but cedar (a softwood) contains a paucity of the terminal β-5 substructures.

KEYWORDS: Lignin; β-5 substructures; pyrolysis−gas chromatography(−mass spectrometry); tetramethylammonium hydroxide (TMAH); dehydrogenation polymer of coniferyl alcohol; Japanese cedar (*Cryptomeria japonica*) wood

INTRODUCTION

Lignin is a biopolymer that contains phenylpropane units connected by C−C and C−O linkages and forms a three-dimensional macromolecular system. Its complicated chemical structures and close association with other biopolymers in cell walls make lignin analysis difficult. Analytical pyrolysis has begun to contribute to the structural elucidation of such intractable polymers (*1*).

Analytical pyrolysis is a technique in which polymers are reproducibly broken down by heat-induced bond dissociation to produce compounds reflecting the structural attributes of the original polymers. However, the pyrograms for lignin often provide less structural information on the substructures. The polar and/or high molecular weight products that are present in the pyrolysates do not have the necessary volatilities to pass through a gas chromatograph (GC) column to be detected (*2*). Therefore, conventional pyrolysis often provides biased pyrograms in favor of monomeric products. Deciphering the lignin structure by use of the pyrolysis data is difficult.

A new pyrolysis procedure has emerged that uses tetramethylammonium hydroxide (TMAH) (*3*). It is widely used to characterize hydrolyzable biopolymers such as lignins (*4*−*13*) and humic acids (*7*, *14*−*17*) with success. More applications of this method are given by Challinor (*18*). This method renders polar pyrolysis products volatile enough to be eluted from the GC column by subsequent on-line methylation. Therefore, we anticipated that dimeric products which retained the structural attributes of the lignin would be displayed in the TMAH/pyrograms of lignins.

Lignin comprises diverse substructures. Our attention was given to the TMAH/pyrolysis products derived from β-5 substructures, accounting for roughly 9−12% of the total interunit linkages in spruce milled wood lignin (*19*) and 45% of the total interunit linkages in a dehydrogenation polymer of coniferyl alcohol (*20*). Substructures containing β-5 linkages are very resistant to pulping. Quantifying their amounts could provide important information on suitable pulpwood types and the effectiveness of pulping processes.

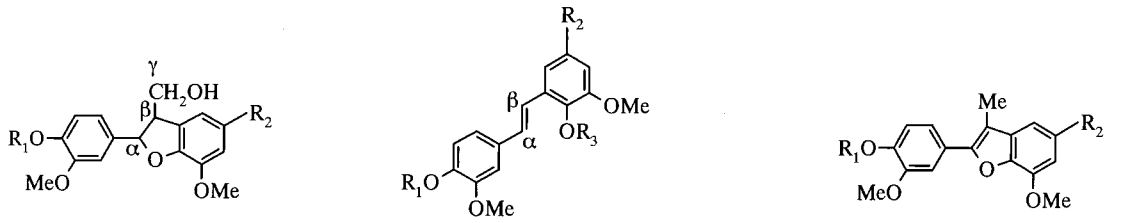
The goal of this paper was to clarify the behavior of the β-5 substructures in TMAH/pyrolysis. Two 2-arylcoumaran type lignin model compounds related to softwood β-5 substructures, dehydrodiconiferyl alcohol (**1**) and its methyl ether **2** (**Figure 1**), were subjected to TMAH/pyrolysis; these compounds represent terminal β-5 substructures in lignin. TMAH/pyrolysis products derived from the model compounds were compared with the pyrolysates of reference lignins.

MATERIALS AND METHODS

Melting points were uncorrected. Elemental analysis was done with a Perkin-Elmer 2400 CHN elemental analyzer. Column chromatography (Wakogel C200, Wako Pure Chemical Industries, Osaka, Japan) was carried out with a benzene−ethyl acetate (15:1, v/v) solvent system.

* Corresponding author. Phone: +81-298-53-4649. Fax: +81-298-55-2203. E-mail: ken@sakura.cc.tsukuba.ac.jp.
† University of Tsukuba.
‡ Institute of Paper Science and Technology.

10.1021/jf011563c CCC: $22.00
Published on Web 05/04/2002



	R_1	R_2 (α′ β′ γ′)
1	H	CH=CHCH$_2$OH
2	Me	CH=CHCH$_2$OH
3	H	CH$_2$CH$_2$CH$_2$OH

	R_1	R_2	R_3
4	H	CH=CHCH$_2$OH	H
5	Ac	CH=CHCH$_2$OAc	Ac
6	Me	CH=CHCH$_2$OMe	Me
7	H	CH$_2$CH$_2$CH$_2$OH	H

	R_1	R_2
8	Me	CH=CHCH$_2$OMe

Figure 1. Chemical structures of compounds **1**–**8**.

Thin-layer chromatography (TLC) was performed on silica gel plates (Merck, Kieselgel 60 F_{254}, 20 μm thick on aluminum sheet) with benzene−ethyl acetate (15:1, v/v) as eluent. Spots were made visible with UV light. ^{1}H Nuclear magnetic resonance (NMR) spectra were obtained on a JEOL EX-270 spectrometer and reported by chemical shifts (relative to tetramethylsilane), splitting patterns, integration areas, and proton assignments. Mass spectrometry (MS) analyses employed an HP 5890 series II gas chromatograph (Hewlett-Packard, Palo Alto, CA) with an HP 5972A quadrupole mass selective detector (Hewlett-Packard), a fused-silica Quadrex MS capillary column (25 m × 0.25 mm i.d.; film thickness, 0.25 μm), column temperature 50 °C (1 min), 5 °C/min to 280 °C (hold), injector temperature 280 °C, detector temperature 280 °C, helium carrier gas at 30 mL/min, a jet separator at 175 °C, a source temperature of 200 °C, and an ionization voltage of 70 eV. Spectra were acquired by an HP ChemStation software package. The mass range used was *m/z* 50−600.

Materials. A 25% TMAH methanolic solution, TMAH pentahydrate, and coniferyl alcohol were commercial products (Aldrich, Milwaukee, WI). Horseradish peroxide was commercially available (Sigma, St. Louis, MO, EC 1.11.1.7, 60 purpurogallin units/mg).

Bulk dehydrogenation polymer of coniferyl alcohol was prepared by one addition of three solutions (*21*): a 0.1 M phosphate buffer solution (200 mL, pH 6.5) containing 1.07 g of coniferyl alcohol, 60 mL of 0.5% hydrogen peroxide, and a 0.1 M phosphate buffer solution (50 mL, pH 6.5) containing 2.5 mg of horseradish peroxidase. The reaction mixture was stirred under Ar atmosphere at room temperature; the polymerization was protected from light by covering the reactor with aluminum foil. After 24 h, the white precipitate was collected, purified, and dried on P_2O_5 (yield 550 mg).

Dehydrogenation polymer of coniferyl alcohol by the dialysis membrane method was prepared according to the method of Tanahashi and Higuchi (*22*). A 0.03 M phosphate buffer solution (10 mL, pH 6.1) containing the peroxidase (5.5 mg) was charged in a cellulose dialysis tube (Wako Pure Chemical Industries, size 36). The five tubes were immersed in a 0.0.3 M phosphate buffer solution (600 mL, pH 6.1) containing coniferyl alcohol (300 mg). Hydrogen peroxide (30%, 0.2 mL) was added to the coniferyl alcohol solution; the polymerization was done in a beaker covered with aluminum foil. After 48 h, with stirring, at room temperature, the white precipitates in the tubes were collected and dried on P_2O_5 (yield 150 mg).

Dehydrodiconiferyl alcohol (**1**) (mp 153−154 °C, lit. (*23*) 155.5−157 °C) was prepared by enzymatic dehydrogenation of coniferyl alcohol with hydrogen peroxide−horseradish peroxidase (*23*). Structure confirmation was provided by NMR and MS; the numbering system of α, β, γ and α′, β′, γ′ is provided in **Figure 1**. ^{1}H NMR (acetate) δ (ppm) 2.06 (3H, s, OCOCH$_3$), 2.10 (3H, s, OCOCH$_3$), 2.31 (3H, s, Ar−OCOCH$_3$), 3.74−3.90 (1H, m, β−CH), 3.81 (3H, s, OCH$_3$), 3.92 (3H, s, OCH$_3$), 4.30 (1H, dd, J = 11.2 and 7.6 Hz, γ_1−CH), 4.46 (1H, dd, J = 11.2 and 5.3 Hz, γ_2−CH), 4.71 (2H, dd, J = 6.6 and 1.0 Hz, γ′−CH$_2$), 5.54 (1H, d, J = 6.6 Hz, α−CH), 6.16 (1H, dt, J = 15.8 and 6.6 Hz, β′−CH), 6.60 (1H, d, J = 15.8 Hz, α′−CH), 6.89−7.03 (5H, m, Ar−H). MS *m/z* (%) 358 (M$^+$, 44), 340 (100), 322 (78), 310 (46), 309 (25), 307 (57), 291 (27), 165 (24), 151 (22), 137 (45).

Compound **2** was prepared by methylating **1** with diazomethane-ether, and used without further purification except simple removal of the solvent in vacuo. ^{1}H NMR (acetate) δ (ppm) 2.06 (s, 3H, OCOCH$_3$), 2.10 (3H, s, OCOCH$_3$), 3.74−3.90 (1H, m, β−CH), 3.81 (3H, s, OCH$_3$), 3.92 (6H, s, OCH$_3$), 4.30 (1H, dd, J = 11.2 and 7.6 Hz, γ_1−CH), 4.46 (1H, dd, J = 11.2 and 5.3 Hz, γ_2−CH), 4.71 (2H, dd, J = 6.6 and 1.0 Hz, γ′−CH$_2$), 5.54 (1H, d, J = 6.6 Hz, α−CH), 6.16 (1H, dt, J = 15.8 and 6.6 Hz, β′−CH), 6.60 (1H, d, J = 15.8 Hz, α′−CH), 6.82−6.96 (5H, m, Ar−H). MS (acetate) *m/z* (%) 456 (M$^+$, 14), 412 (11), 396 (44), 352 (45), 327 (59), 167 (38), 165 (90), 151 (100), 117 (78), 73 (35).

Acetate **5** was obtained by reacting **1** (200 mg) with 1 N sodium hydroxide (10 mL) for a week at room temperature, with stirring, under Ar atmosphere. After acidification of the reaction mixture with acetic acid, the brown precipitates obtained were washed with water. The air-dried precipitates were acetylated by a mixture of acetic anhydride and pyridine (5 mL, 1:1, v/v) overnight at room temperature. Pouring the reaction mixture into ice−water provided light brown precipitates. Column chromatography of the precipitates (160 mg) with benzene−ethyl acetate (15:1, v/v) as eluant gave an oil. Crude crystals (40 mg) with a 0.19 R_f were obtained from ethyl alcohol. Recrystallization (2×) provided colorless crystals (27 mg, ethyl alcohol, 94% GC−MS pure) of mp 148−149 °C (Found: C, 65.1; H, 5.80. $C_{25}H_{26}O_8$ requires C, 66.1; H, 5.76%). ^{1}H NMR (CDCl$_3$) δ (ppm) 2.12 (3H, s, OCOCH$_3$), 2.33 (3H, s, Ar−OCOCH$_3$), 2.37 (3H, s, Ar−OCOCH$_3$), 3.86 (3H, s, OCH$_3$), 3.88 (3H, s, OCH$_3$), 4.75 (2H, d, J = 6.3 Hz, γ′−CH$_2$), 6.29 (1H, dt, J = 15.8 and 6.3 Hz, β′−CH), 6.65 (1H, d, J = 15.8 Hz, α′−CH), 6.92−7.10 (6H, m, Ar−H + vinyl−H), 7.25 (1H, d, J = 2.6 Hz, Ar−H). MS *m/z* (%) 454 (M$^+$, 24), 412 (M$^+$ − COCH$_2$, 35), 370 (M$^+$ − 2 × COCH$_2$, 48), 310 (370 − CH$_3$COOH, 100), 281 (19), 207 (44).

On-line methylation of pinoresinol, prepared by enzymatic dehydrogenation of coniferyl alcohol with hydrogen peroxide−horseradish peroxidase (*23*), with TMAH provided its methyl ether **12**: MS *m/z* (%) 386 (M$^+$, 40), 177 (70), 165 (100), 151 (80).

TMAH/Pyrolysis−GC−MS. The pyrolysis−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 50μm-ferromagnetic pyrofoil. The 25% TMAH methanolic solution (∼2 μL) was added to compounds **1** and **2** (∼10 μg) on the pyrofoil with a syringe. Similarly, the 25% TMAH methanolic solution (5 μL) was added to the dehydrogenation polymers (∼50−70 μg); TMAH pentahydrate (∼1 mg) was used in TMAH/pyrolysis of a Japanese cedar (*Cryptomeria japonica*) wood (∼100−200 μg). The mixture was tightly wrapped. 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 500 °C for 4 s under a flow of helium carrier gas; our Curie-point pyrolysis system heats the pyrofoil to 500 °C in about 0.2−0.3 s. The volatile products were sent to the GC−MS and subjected to the MS analyses. Identification

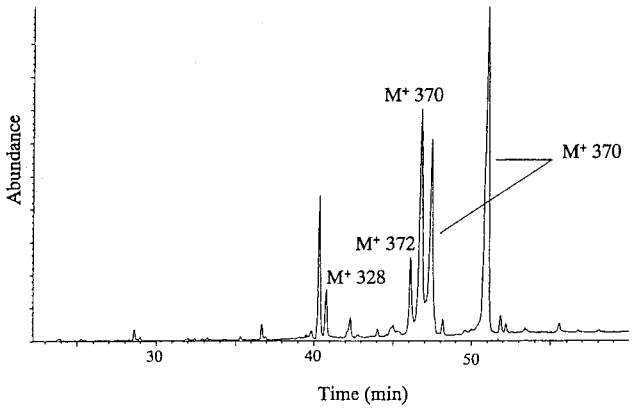


Figure 2. Partial TMAH/pyrolysis–GC–MS trace of **1**.

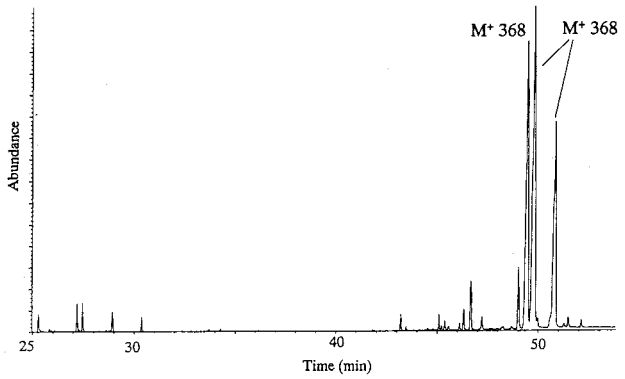


Figure 3. Partial TMAH/pyrolysis–GC–MS trace of **2**.

of the products was done by comparing the retention times of standard compounds or their on-line TMAH/methylation products (*24*) with those of the products, and by comparing the MS data of the products with published data (*9*, *24*).

TMAH/Pyrolysis−GC. The TMAH/pyrolysis−GC system was a combination of a Curie-point pyrolyzer (JHP-3 model, Japan Analytical Industry) and a Shimadzu GC-17A gas chromatograph (Shimadzu, Kyoto, Japan) with a flame ionization detector and a 1:50 split ratio injector. The TMAH/pyrolysis−GC runs were done similarly to the TMAH/pyrolysis−GC−MS runs. Product identification was done based on the TMAH/pyrolysis−GC−MS results.

On-Line Methylation with TMAH. The 25% TMAH methanolic solution containing the sample was introduced into the GC injection port heated at 300 °C, and the product analysis was done according to the TMAH/pyrolysis−GC−MS results.

RESULTS AND DISCUSSION

TMAH/Pyrolysis of 2-Arylcoumaran Type Lignin Model Compounds. The chemical structures of compounds **1**−**8** are shown in **Figure 1**. **Figure 2** shows the partial TMAH/pyrolysis−GC−MS trace of **1**. Three main products were obtained in a 3:3:5 GC−MS signal area ratio (order of GC−MS elution time). However, no signals with the molecular ion of 400 (permethylated product of **1**) were observed. The observed signals had the same molecular ion (M^+ 370) and a similar MS fragmentation pattern: MS (for the former two) *m*/*z* (%) 371 ($M^+ + 1$, 24), 370 (M^+, 100), 207 (3), 165 (11), 151 (15), 71 (90); MS (for the latter) *m*/*z* (%) 371 ($M^+ + 1$, 24), 370 (M^+, 100), 207 (10), 165 (20), 151 (21), 71 (20).

TMAH/pyrolysis proceeds under basic conditions. Therefore, the behavior of 2-arylcoumaran type lignin model compounds in hot alkaline media, such as soda pulping, helped identify the M^+ 370 product. In hot alkaline media, phenolic α-aryl ether linkages are opened to form a new phenolic group and lose the γ-CH_2OH group (if present) as formaldehyde; final products are 2,4′-dihydroxystilbenes (*25*−*29*). In a related study, Miksche (*30*) showed that mild alkaline treatment of dihydrodehydrodiconiferyl alcohol (**3**) ($R_2 = -CH_2CH_2CH_2OH$ instead of $-CH{=}CHCH_2OH$ of **1**) provides stilbene **7**, which has two phenolic hydroxyl groups, and loses the γ-CH_2OH substituent, similar to kraft and soda processes. If a similar process occurred during TMAH/pyrolysis the stilbene products in our case would give, after methylation, a M^+ 370 product.

To identify the M^+ 370 product, we subjected **1** to a mild alkaline treatment, which should minimize degradation and/or polymerization of the cinnamyl group in the hot alkaline solution. Compound **1** was stirred in 1 N sodium hydroxide solution for a week at room temperature; subsequent acetylation provided a crystalline acetate product in ca. 10% yield, after chromatographic purification and two recrystallizations. The ^1H NMR analysis of the acetate showed a singlet signal (3H) at 2.12 ppm due to an alcoholic acetyl group, and two singlet signals (each 3H) at δ 2.33 and 2.37 due to two phenolic acetyl groups. The signals at δ 4.75, 6.29, and 6.65 (ascribed to the γ′, β′, and α′-side chain protons, respectively) demonstrate the presence of the (*E*)-Ar−CH=CH−CH_2O− moiety; therefore, the cinnamyl alcohol moiety survived the alkaline treatment. These results demonstrate that the alkali treatment cleaved the hydrofuran ring of **1** to generate a phenol, and removed the γ-hydroxymethyl substituent as HCHO, similar to the reaction of **3** with alkali. On the basis of the reported literature of the behavior of phenolic 2-arylcoumarans in alkaline media (*25*−*30*), and the NMR results on the acetate, we assigned the structure to the triacetate (**5**) of 2,4′-dihydroxy-3,3′-dimethoxy-5-(3-hydroxyprop-1-enyl)stilbene (**4**). The molecular ion at *m*/*z* 454, and the fragment ions at *m*/*z* 412, 370 and 310, due to the release of three acetyl groups, supported the structure of **5**. The analytical data did not specify the configuration of stilbene **5**. However, the hot (*26*) and cold (*30*) alkaline treatments of **3** give *trans*-stilbene **7** in 76 and 88% yields, respectively. These results suggest that **5** also favors a *trans*-form, although the yield was low; the acetate contained 6% *cis*-isomer on the basis of the GC−MS analysis.

Acetate **5** was on-line methylated with TMAH to confirm whether **5** is a precursor for the M^+ 370 products. Three large signals having M^+ 370 were observed in a 13:10:77 GC−MS signal area ratio (order of GC−MS elution time), along with small levels of products having the molecular ions at *m*/*z* 372 and 328. The 84 Da decrease (454 → 370) indicates that TMAH/pyrolysis hydrolyzed the all acetyl groups and substituted methyl groups for them. Each of the retention times and the MS fragmentation patterns of the M^+ 370 product isomers obtained from **5** was identical with that of the M^+ 370 product isomers obtained from **1**, although the isomer distribution profile differed from that obtained from **1**. On the basis of these findings, we assign the M^+ 370 products as 2,3,3′,4′-tetramethoxy-5-(3-methoxyprop-1-enyl)stilbene (**6**) and its isomers. Despite the use of **5** with a high purity (a 94% GC−MS pure), three isomers were obtained, suggesting that isomerization occurs during TMAH/pyrolysis. There are four possible isomers related to cis/trans configurations of the stilbene and side chain double bonds.

Nonphenolic α-aryl ether linkages are reported to be stable during kraft and soda pulping conditions (*25*, *27*−*29*). The hydrofuran ring is not cleaved to generate a new phenolic hydroxyl group; the model compounds were recovered in quantitative yields from the hot alkaline solutions (*25*, *28*). **Figure 3** shows the partial TMAH/pyrolysis−GC−MS trace of **2**. Compound **2** produced neither the permethylated products with the M^+ 400 nor **6**. As shown in **Figure 3**, **2** provided three

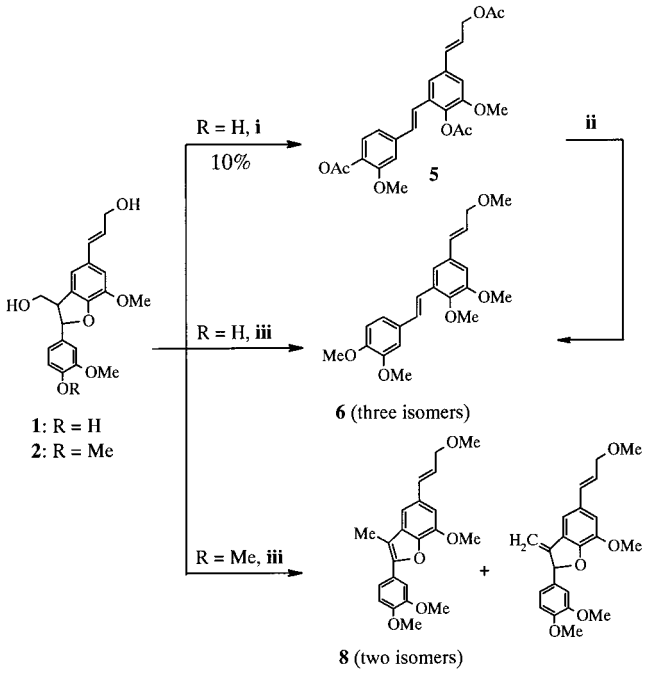


Figure 4. Schematic formation of **5**, **6**, and **8** from 2-arylcoumarans **1** and **2**: (i) 1 N NaOH, 1 week, Ar, r.t./acetylation with Ac_2O–pyridine/column chromatography/recrystallization; (ii) on-line methylation with TMAH; (iii) TMAH/pyrolysis at 500 °C for 4 s.

main products in a 77:100:60 GC−MS signal area ratio (order of GC−MS elution time). These were assigned to be isomers because they have the same molecular ion (M^+ 368) and similar MS fragmentation patterns: MS *m*/*z* (%) 369 ($M^+ + 1$, 23), 368 (M^+, 100), 353 (25), 337 (16), 325 (12), 165 (16). The finding, the survival of the cinnamyl group like **6** and no cleavage of the hydrofuran ring, suggests that the products are best accommodated by 2-arylbenzofuran **8** and its isomers. That is, TMAH/pyrolysis dehydrates the γ-OH group and the β-proton in **2** to generate the benzofuran ring simultaneously. Unlike **1**, which lost the γ-substituent, **2** retained the γ-carbon as a methyl group and possibly a methylene ($=CH_2$) group. Structure **8** should have only two isomers (cis/trans with the side chain double bond); the observed third isomer might represent a $=CH_2$ unit. **Figure 4** summarizes the formation of **5**, **6**, and **8** from 2-arylcoumarans **1** and **2**.

TMAH/Pyrolysis of Synthetic and Natural Lignins. To learn the contribution of the β-5 substructures in lignin pyrolysates, we pyrolyzed synthetic and cedar native lignins in the presence of TMAH. **Figure 5** shows the TMAH/pyrolysis−GC trace of the bulk dehydrogenation polymer of coniferyl alcohol; assignment of the products was done by TMAH/pyrolysis−GC−MS. A monomeric product region at <40 min retention time displays the signals of coniferyl alcohol dimethyl ether (**9**) and *erythro*/*threo*-1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane (**10**/**11**), accounting for 13 and 21%, respectively, of the integrated GC signal areas between 20 and 60 min retention time. The former is a product stemming from cleavage of coniferyl alcohol-end groups (*31*) and the latter is from cleavage of β-aryl ether substructures (*12*, *13*).

Signals ascribed to **6** were observed in a 1:0.5:1 GC signal area ratio (order of GC elution time) between 50.5 and 52.5 min retention time, along with a large level of pinoresinol dimethyl ether (**12**). Product **6** contributed ~15.8% to the sum of the integrated GC signal areas between 20 and 60 min retention time, and ~40% to a dimeric product region (retention times of 40−60 min). Likewise with the bulk polymer, the dialysis dehydrogenation polymer also yielded a large level of **6** (the pyrogram is not shown). On the basis of the relative GC signal areas, we concluded from our results that synthetic lignins contain a significant amount of β-5 terminal substructures.

TMAH/pyrolysis−GC of cedar wood provided a very small amount of **6** (the pyrogram is not shown). Assuming that the cedar wood we examined is similar to other softwoods, which reportedly contain 9−12% β-5 substructures (*19*), we are led

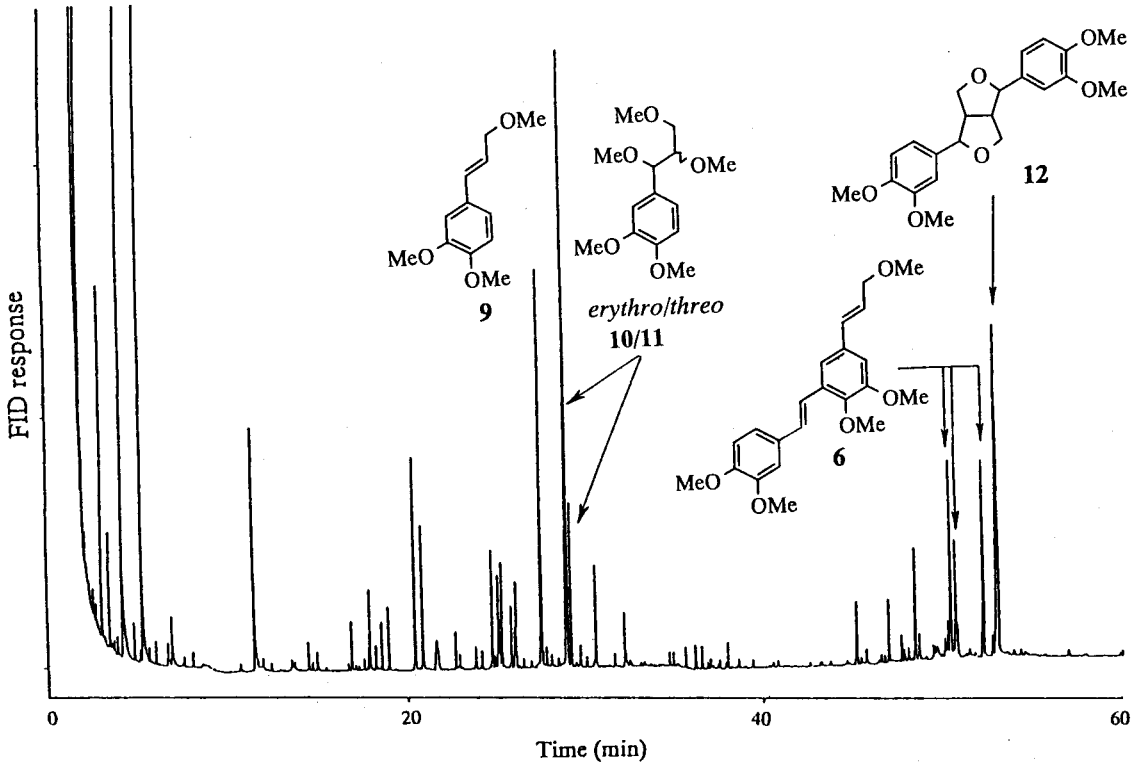


Figure 5. TMAH/pyrolysis–GC trace of bulk dehydrogenation polymer of coniferyl alcohol.

to conclude that most of the β-5 substructures in cedar (softwood) lignin are present in the lignin by way of linking with more than one lignin substructure. Such β-5 substructures will likely not give volatile, characteristic products when lignin is subjected to TMAH/pyrolysis−GC(−MS) analysis.

In summary, TMAH/pyrolysis of phenolic models and lignin end units that have β-5 substructures provides methylated stilbene products that are easily observable by GC(−MS). Synthetic lignins show high levels of such structures, whereas cedar wood, a softwood, shows practically none. Nonphenolic β-5 lignin model compounds show no simple stilbene products in TMAH/pyrolysis.

ACKNOWLEDGMENT

We thank the Chemical Analysis Center, University of Tsukuba, for chemical and ^{1}H NMR spectral analysis data.

LITERATURE CITED

(1) Meier, D.; Faix, O. Pyrolysis−Gas Chromatography−Mass Spectrometry. In *Methods in Lignin Chemistry*; Lin, S. Y., Dence, C. W., Eds.; Springer-Verlag: Berlin-Heidelberg, Germany, 1992; pp 177−199.

(2) Izumi, A.; Kuroda, K. Pyrolysis−mass spectrometry analysis of dehydrogenation polymers with various guaiacyl/syringyl ratios. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1405−1411.

(3) 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.

(4) McKinney, D. E.; Carson, D. M.; Clifford, D. J.; Minard, R. D.; Hatcher, P. G. Off-line thermochemolysis versus flash pyrolysis for the in situ methylation of lignin: is pyrolysis necessary? *J. Anal. Appl. Pyrolysis* **1995**, *34*, 41−46.

(5) 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.

(6) 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.

(7) 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.

(8) González-Vila, F. J.; Almendros, G. C.; del Rio, J. C.; Martin, F.; Gutiérrez, A.; Romero, J. Ease of delignification assessment of wood from different *Eucalyptus* species by pyrolysis (TMAH)−GC/MS and CP/MAS ^{13}C NMR spectrometry. *J. Anal. Appl. Pyrolysis* **1999**, *49*, 295−305.

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

(10) 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.

(11) Vane, C. H.; Abbott, G. D.; Head, I. M. The effect of fungal decay (*Agaricus bisporus*) on wheat straw lignin using pyrolysis−GC−MS in the presence of tetramethylammonium hydroxide (TMAH). *J. Anal. Appl. Pyrolysis* **2001**, *60*, 69−78.

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

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

(14) Martin, F.; del Rio, J. C.; González-Vila, F. J.; Verdejo, T. Pyrolysis derivatization of humic substances. 2. Pyrolysis of soil humic acids in the presence of tetramethylammonium hydroxide. *J. Anal. Appl. Pyrolysis* **1995**, *31*, 75−83.

(15) Lehtonen, T.; Peuravuori, J.; Pihlaja, K. Characterisation of lake-aquatic humic matter isolated with two different sorbing solid techniques: tetramethylammonium hydroxide treatment and pyrolysis−gas chromatography/mass spectrometry. *Anal. Chim. Acta* **2000**, *424*, 91−103.

(16) Mansuy, L.; Bourezgui, Y.; Garnier-Zarli, E.; Jardée, E.; Réveillé, V. Characterization of humic substances in highly polluted river sediments by pyrolysis methylation−gas chromatography−mass spectrometry. *Org. Geochem.* **2001**, *32*, 223−231.

(17) Hatcher, P. G.; Dria, K. J.; Kim, S.; Frazier, S. W. Modern analytical studies of humic substances. *Soil Sci.* **2001**, *166*, 770−794.

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

(19) Adler, E. Lignin Chemistry − Past, Present and Future. *J. Wood Sci. Technol.* **1977**, *11*, 169−218.

(20) Landucci, L. L. Reaction of *p*-hydroxycinnamyl alcohols with transition metal salts. 1. Oligolignols and polylignols (DHPs) from coniferyl alcohol. *J. Wood Chem. Technol.* **1995**, *15*, 349−368.

(21) Shevchenko, S. M.; Akim, L. G.; Tanahashi, M.; Higuchi, T. Comparative study on mild depolymerization of lignin model dehydrogenation polymers and milled wood lignin. *J. Wood Chem. Technol.* **1995**, *15*, 163−178.

(22) Tanahashi, M.; Higuchi, T. Dehydrogenative polymerization of monolignols by peroxidase and H_2O_2 in a dialysis tube. I. Preparation of highly polymerized DHPs. *Wood Res.* **1981**, *67*, 29−42.

(23) Katayama, Y.; Fukuzumi, T. Enzymatic synthesis of three lignin-related dimers by an improved peroxidase−hydrogen peroxide system. *Mokuzai Gakkaishi* **1978**, *24*, 664−667.

(24) Kuroda, K. Electron-impact (EI) mass spectra of 1,2-dimethoxybenzenes related to the pyrolysis products of guaiacyl lignin in the presence of tetramethylammonium hydroxide (TMAH). *J. Anal. Appl. Pyrolysis;* in press

(25) Gierer, J.; Noren, I. Über die Reaktionen des Lignins bei der Sulfatkochung II. Modellversuche zur Spaltung von Aryl−alkylätherbindungen durch Alkali. *Acta Chem. Scand.* **1962**, *16*, 1713−1729.

(26) Adler, E.; Marton, J.; Falkehag, I. The behaviour of lignin in alkaline pulping. I. Model experiments with phenylcoumarans. *Acta Chem. Scand.* **1964**, *18*, 1311−1312.

(27) Wallis, A. F. A. The alkaline hydrolysis of some lignin model 2-arylcoumarans. *Aust. J. Chem.* **1972**, *25*, 1529−1535.

(28) Gierer, J.; Lenz, B.; Wallin, N.-H. The reactions of lignin during sulphate cooking. Part V. Model experiments on the splitting of aryl−alkyl ether linkages by 2 N sodium hydroxide and by white liquor. *Acta Chem. Scand.* **1964**, *18*, 1469−1476.

(29) Wacek, A. v. u.; Voykowitsch, A. Die alkalische Hydrolyse von Dehydrodiisoeugenol unter verschiedenen Aufschlußbedingungen. *Holzforschung* **1968**, *22*, 77−80.

(30) Miksche, G. E. Über das Verhalten des Lignins bei der Alkalikochung VI. Zum Abbau von *p*-Hydroxy-phenylcumaran-strukturen durch Alkali. *Acta Chem. Scand.* **1972**, *26*, 3269−3274.

(31) Kuroda, K.; Nakagawa-izumi, A. Characterization of lignin by analytical pyrolysis in the presence of tetramethylammonium hydroxide. *Proceedings of the 10th International Symposium on Wood and Pulp Chemistry*, Japan Tappi: Yokohama, Japan, 1999; pp132−135.

Received for review November 27, 2001. Revised manuscript received March 19, 2002. Accepted March 19, 2002.

JF011563C