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Pyrolysis of Lignin in the Presence of Tetramethylammonium Hydroxide: A Convenient Method for S/G Ratio Determination

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Pyrolysis−gas chromatography in the presence of tetramethylammonium hydroxide (TMAH) was applied to the determination of the ratio of the abundances of the syringyl β-aryl ether subunits to those of the guaiacyl equivalents (S/G) in lignin. Diazomethane-methylated kenafs (*Hibiscus cannabinus* and *Hibiscus sabdariffa*) and beech (*Fagus crenata*) in situ lignins were employed. Relative abundances of pyrolysis products derived from the guaiacyl and syringyl β-aryl ether subunits were determined. The S/G ratios for in situ lignins were obtained with average 3.1% relative standard deviation for a minimum of six repeated runs. The S/G ratios determined by pyrolysis in the presence of TMAH agreed well with those determined by thioacidolysis, with a significant linear regression ($R^2 = 0.9867$). The results showed that pyrolysis with TMAH is an effective tool for obtaining information on the S/G ratio for in situ lignins.

KEYWORDS: Lignin; pyrolysis−gas chromatography (−mass spectrometry); tetramethylammonium hydroxide; thioacidolysis; nitrobenzene oxidation; β-aryl ether subunits; kenaf (*Hibiscus cannabinus* and *Hibiscus sabdariffa*); beech (*Fagus crenata*)

INTRODUCTION

Lignin is an important constituent of biomass. In vascular plants, lignin comprises 20−35 wt % of the biomass, and in herbaceous plants, it comprises 15−25 wt % of the biomass. Lignins are built up of randomly cross-linked phenylpropane type subunits. The most frequent linkage between subunits is a β-aryl ether linkage (48 and 60% in spruce and birch lignins, respectively (*1*)). Because of its dominance and great reactivity during pulping and bleaching processes, the β-aryl ether linkage has been the focus of many studies. In particular, selective and efficient methods for analyzing β-aryl ether subunits have taken on greater importance.

Thioacidolysis (*2*−*4*) and derivatization followed by reductive cleavage, the so-called DFRC method (*5*), have gained wide acceptance as effective tools for analyzing the amounts of β-aryl ether linkages in biomass samples. However, these methods require large amounts of sample (>mg range), odiferous chemicals, and multistep sample preparation prior to chromatographic analyses.

The combination of pyrolysis with high-resolution capillary gas chromatography (GC) and mass spectrometry (MS) (pyrolysis−GC−MS) has been increasingly used for the determination of the chemical compositions of lignins in wood chemistry (*6*). The rapid analysis employs a simple sample preparation (drying and milling), small sample sizes (100−200 μg range), and routine identification of the major pyrolysis products (*6*−*15*). However, the specificity of the method is low because it randomly destroys the subunits in lignin. Therefore, a typical pyrolysis−GC−MS generally provides less structural information than the other methods.

Pyrolysis in the presence of tetramethylammonium hydroxide (TMAH), developed by Challinor (*16*), is a novel technique that has provided detailed information on both the structure and the composition of polymers. A vast body of TMAH/pyrolysis data has been accumulated in the area of lignin and humic acids; many products such as **1**−**14** are reported (*14*, *17*−*20*) (**Figure 1**). Recent studies (*18*−*23*) have established that this method fragments lignin by selective cleavage of the β-aryl ether linkages, similar to thioacidolysis. Thioacidolysis products **15**/**16** and **17**/**18** carry quantitative information on noncondensed guaiacyl and syringyl β-aryl ether linkages in lignin (**Figure 2**). An open question is whether the same is the case for the TMAH/pyrolysis products. Unlike conventional pyrolysis (without TMAH), whose data correlate well with compositional data obtained by wet chemical methods (*10*−*12*), there are no published reports of a systematic comparison of analytical TMAH/pyrolysis with established techniques, although the determination of the lignin composition by this method was already carried out on *Eucalyptus* woods (*23*).

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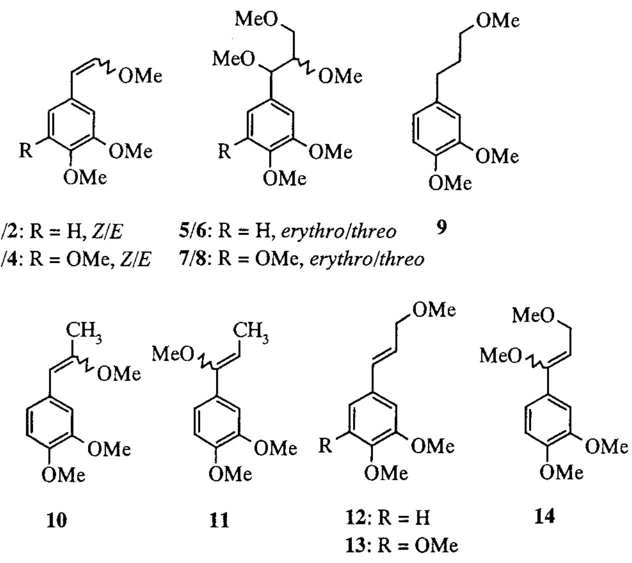

Figure 1. Selected TMAH/pyrolysis products (*14*, *17–20*).

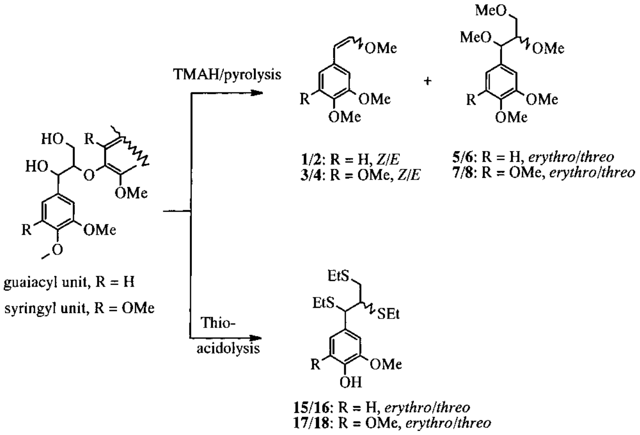

Figure 2. Main products derived from the cleavage of β-aryl ether linkages by TMAH/pyrolysis and thioacidolysis. The names for products **1**–**8** are provided in **Table 2**.

We are presently exploring the feasibility of TMAH/pyrolysis as a practical technique for characterizing lignin. Research efforts were directed at clarifying the correlation of TMAH/pyrolysis results with thioacidolysis results. For this purpose, samples with different ratios of syringyl β-aryl ether linkages to guaiacyl β-aryl ether linkages (S/G) were analyzed by both TMAH/pyrolysis and thioacidolysis. Such efforts will extend the possibility of analytical pyrolysis.

MATERIALS AND METHODS

Materials. Details of kenafs employed are described in a previous paper (*24*). Kenafs, varieties Chinpi-3 (*Hibiscus cannabinus*) and Mesta (*Hibiscus sabdariffa*), were planted in May 1998 and harvested in January 1999 in Kochi, Japan. The Chinpi-3 variety provided samples A (core) and F (bast); the Mesta variety provided samples B (core), D (inner bast), and E (bast). As a wood sample, beech (*Fagus crenata*, sample C) was used. All samples were ground; the materials that passed through a 60−80 mesh screen were Soxhlet-extracted with ethanol:benzene (1:2, v/v) for 6 h. After the samples were air-dried, the extractive-free samples were subjected to the lignin analysis. For TMAH/pyrolysis, diazomethane-methylated samples were used.

(1*E*)-2-(3,4-Dimethoxyphenyl)-1-methoxyethene (**2**) was synthesized by heating 4-((1*E*)-2-bromovinyl)-1,2-dimethoxybenzene (*25*) with sodium methoxide at 170 °C for 4 h (*26*). ^{1}H NMR ($CDCl_3$): δ 3.73 (3H, s, OCH_3), 3.83 (3H, s, Ar−OCH_3), 3.86 (3H, s, Ar−OCH_3), 5.14 (1H, d, J = 7.3 Hz, vinyl-H), 6.03 (1H, d, J = 6.9 Hz, vinyl-H), 6.77 (1H, d, J = 8.2 Hz, Ar−H), 7.06 (1H, dd, J = 8.2, 2.0 Hz, Ar−H), 7.23 (1H, d, J = 2.0 Hz, Ar−H). MS (*m*/*z*, %) data are given in **Table 2**. *erythro*/*threo*-1,2-Dimethoxy-4-(1,2,3-trimethoxypropyl)benzene (**5**/**6**) were prepared by injecting a mixture of the corresponding 3,4-dimethoxyphenylglycerols (*25*) and a 25% TMAH methanolic solution into a GC injection port heated at 300 °C; MS (*m*/*z*, %) data are given in **Table 2**.

erythro-1-(3,4-Dimethoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol (**19**) was prepared by methylating *erythro*-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol (Tokyo Chemical Industry, Tokyo, Japan) with diazomethane/ether and was used without further purification except simple distillation in vacuo of the solvent at <40 °C.

3-Ethoxy-4-hydroxybenzaldehyde, boron trifluoride diethyl ether complex (BF_3 etherate), hexacosane, and ethanethiol were commercial products (Wako Chemical Industry, Tokyo, Japan). TMAH$\cdot 5H_2O$ and the 25% TMAH methanolic solution were supplied from Aldrich. *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was a commercial product (Tokyo Chemical Industry).

Lignin Determination. The lignin content was determined in the extractive-free sample by the modified 72% sulfuric acid method (*27*). Triplicate runs were done for each sample. The relative standard deviation (RSD), determined by dividing the standard deviation by the mean value, was 1.3% with sample A.

Nitrobenzene Oxidation. A mixture of the sample (corresponding to ~10 mg of lignin), 4 mL of 2 N NaOH, and nitrobenzene (0.24 mL) in a 10 mL stainless steel pressure vessel was heated at 170 °C for 2 h under continuous shaking. The oxidation products were trimethylsilylated with BSTFA/pyridine prior to the GC analysis. 4-Hydroxy-3-ethoxybenzaldehyde was used as an internal standard. The trimethylsilylated derivatives obtained were analyzed by GC. The GC conditions employed a model GC14-A gas chromatograph (Shimadzu, Kyoto, Japan), equipped with a flame ionization detector, a fused-silica capillary column (Quadrex MS, 25 m × 0.25 mm i.d.; film thickness, 0.25 μm), helium as a carrier gas, an injection temperature of 200 °C, and a detector temperature of 270 °C. The column temperature program was 60−200 °C at 5 °C/min, kept at 200 °C for 5 min, and increased to 270 °C at 10 °C/min, after which it was isothermal for 9 min. Triplicate runs were done for each sample. The RSD was 3.1% with sample A.

Thioacidolysis. The samples (10 mg) were subjected to thioacidolysis according to Lapierre's procedure (*4*). The thioacidolysis products were trimethylsilylated with BSTFA/pyridine. The trimethylsilylated monomeric products were identified by GC−MS and quantified by GC. The GC conditions employed were similar to those in the nitrobenzene oxidation runs except that the injection temperature was 280 °C, the detector temperature was 280 °C, and the column temperature program was from 100 to 280 °C at 5 °C/min. Hexacosane was used as an internal standard. Triplicate runs were done for each sample. The RSD was 1.5% with sample A.

TMAH/Pyrolysis−GC. The pyrolysis−GC system was a combination of a JHP-3 model Curie-point pyrolyzer (Japan Analytical Industry, Tokyo, Japan) and a GC-17A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector. The samples (about 100−200 μg) weighed to 0.1 μg accuracy were placed on a 50 μm ferromagnetic pyrofoil and covered with solid TMAH$\cdot 5H_2O$ (ca. 1 mg); a 25% TMAH methanolic solution (2 μL, Aldrich) was added to **19** (~10 μg). The mixture was tightly wrapped in the pyrofoil. The sample-loaded pyrofoil was inserted into a quartz sample tube. The sample holder with the sample tube was centered in the pyrolyzer heated at 250 °C. After a 15 s flushing of the system with helium, the samples were pyrolyzed at 500 °C for 4 s. The pyrolysis temperature of 500 °C was reached in about 300 × 10^{-3} s. The volatile products were sent to the GC via a transfer line heated at 250 °C and separated on a fused-silica capillary column (Quadrex MS, 25 m × 0.25 mm i.d.; film thickness, 0.25 μm). The column temperature was kept at 50 °C for 1 min and ramped at 5 °C/min to 300 °C, after which it was isothermal for 10 min. Helium was used as the carrier gas (flow rate, 1.3 mL/min). The injection and detector ports were kept at 280 °C. A minimum of six repeated TMAH/pyrolyses were done for one sample.

Table 1. Sample Description

					TMAH/pyrolysis			
					product contribution (relative mol %)[d]			
sample	variety, position, fraction	lignin (%)[a]	S/V[b]	thioacidolysis S/G[c]	guaiacyl type[e]	syringyl type[f]	S/G[g,h]	RSD[i]
A	Chinpi-3, upper middle, core	23.5	0.83	1.35	44.8	55.2	1.23 ± 0.03	2.4 (10)[j]
B	Mesta, upper middle, core	22.5	1.63	1.77	38.4	61.6	1.60 ± 0.05	3.1 (10)
C	beech	24.9	2.40	2.02	32.6	67.4	2.07 ± 0.05	2.4 (10)
D	Mesta, bottom, inner bast	22.7	2.04	2.35	30.3	69.6	2.30 ± 0.09	3.9 (6)
E	Mesta, bottom, bast	22.9	2.21	2.60	26.0	74.0	2.85 ± 0.10	3.5 (10)
F	Chinpi-3, upper middle, bast	14.8	2.91	3.72	20.4	79.6	3.89 ± 0.13	3.3 (10)

[a] Klason lignin + acid-soluble lignin. [b] S/V molar ratio by nitrobenzene oxidation. [c] Syringyl β-aryl ether subunits/guaiacyl β-aryl ether subunits ratio by thioacidolysis. [d] Determined on the basis of the GC signal areas and the effective carbon numbers (*14*, *39*). [e] Summed contribution of products **1** + **2** + **5** + **6**. [f] Summed contribution of products **3** + **4** + **7** + **8**. [g] Ratio of **1** + **2** + **5** + **6**/**3** + **4** + **7** + **8**. [h] Expressed as mean value ± standard deviation for repeated runs. [i] RSD (%). [j] Values in parentheses mean the number of repeated runs.

Table 2. Identified TMAH/Pyrolysis Fragments Derived from the β-Aryl Ether Subunits

signal no.	product	*m/z* (relative intensity %)	origin[a]
1	(*Z*)-2-(3,4-dimethoxyphenyl)-1-methoxyethene	194 (M$^+$, 100), 179 (65), 151 (40), 148 (14), 121 (13)	G
2[b]	(*E*) isomer of 1	same as the MS data of 1	G
3	(*Z*)-2-(3,4,5-trimethoxyphenyl)-1-methoxyethene	224 (83), 209 (100), 181 (22), 178 (12), 151 (28)	S
4	(*E*) isomer of 3	same as the MS data of 3	S
5[b]	*erythro*-1,2-dimethoxy-4-(1,2,3-trimethoxypropyl)benzene	270 (M$^+$, 2), 181 (100)	G
6[b]	*threo* isomer of 5	same as the MS data of 5	G
7	*erythro*-1,2,3-trimethoxy-4-(1,2,3-trimethoxypropyl)benzene	300 (M$^+$, 2), 211 (100)	S
8	*threo* isomer of 7	same as the MS data of 7	S

[a] G, guaiacyl β-aryl ether subunits; S, syringyl β-aryl ether subunits. [b] Identified by comparison with the MS data and retention times of authentic compound; other products were identified on the basis of the MS fragment patterns.

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 GC (Hewlett-Packard, Palo Alto, CA) with an HP 5972A quadrupole mass selective detector (Hewlett-Packard). The pyrolyses were similar to the TMAH/pyrolysis−GC runs. Spectra were acquired by an HP ChemStation software package. Peak identification was carried out at a 70 eV electron impact voltage in an ion chamber heated at 200 °C. The mass range scanned was *m/z* 70−600. Peak assignments were carried out on the basis of mass fragmentation patterns and by comparing the MS data with those of authentic samples (*28*).

RESULTS AND DISCUSSION

Sample Description. Table 1 shows the descriptions of the samples employed. Thioacidolysis of samples A−F indicated a 1.35−3.72 range of the S/G ratios. The S/G ratios were determined by calculating the molar yield ratio of **17** + **18** to that of **15** + **16**; products **15** and **16** come from the cleavage of guaiacyl β-aryl ether subunits, and products **17** and **18** come from the cleavage of syringyl equivalents (**Figure 2**). The thioacidolysis values were comparable with the ratios of the syringaldehyde to vanillin molar yield (S/V) obtained by nitrobenzene oxidation; however, except for beech, the latter method gave lower ratios by a factor of 10−40%. The nitrobenzene oxidation products come from a variety of different subunits that are not C_5-substituted in lignin, unlike thioacidolysis products that mostly come from the β-aryl ether subunits.

TMAH/Pyrolysis of a β-Aryl Ether Lignin Model. To confirm the nature of the products related to β-aryl ether subunits, we performed TMAH/pyrolysis of a lignin model compound that contained a β-aryl ether linkage. **Figure 3** shows the TMAH/pyrogram of *erythro*-**19**. Cleavage of the β-guaiacyl ether linkage produced a large amount of 1,2-dimethoxybenzene at a retention time of 10 min. Its counterparts, **1**, **2**, and **5**, which stem from the veratrylglycerol moiety of **19**, were also observed in large amounts. Product **5** (erythro form) retains the structural attribute of the original erythro form of the starting material, suggesting that TMAH/pyrolysis could provide information on the erythro/threo ratio of the β-aryl ether subunits in nature and isolated lignins. The present methods of determining erythro/threo ratios include NMR (*29*) and ozonation (*30*). At the retention time of >38 min, the permethylated product of **19**, 1-[2-(3,4-dimethoxyphenyl)-2-methoxy-1-(methoxymethyl)-ethoxy]-2-methoxybenzene, was produced; two isomers were observed in a 3:1 GC−MS signal area ratio. MS *m/z* (%): 362 (M$^+$, 2), 181 (100), 166 (7), 151 (7).

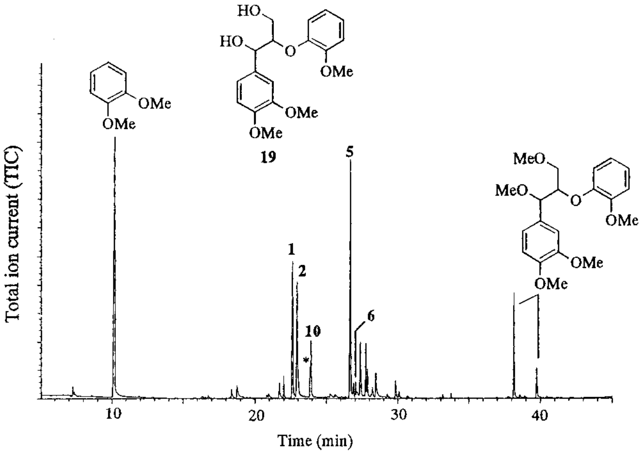

Figure 3. TMAH/pyrolysis–GC–MS trace of **19**. The names and the MS data for products **1**, **2**, **5**, and **6** refer to those in **Table 2**. The asterisk denotes the isomer of **10**.

The GC−MS signal areas of products **1**, **2**, **5**, and **6** relative to the 1,2-dimethoxybenzene signal area were 0.34, 0.25, 0.59, and 0.03, respectively. Our TMAH/pyrolysis product profile for the β-aryl ether type model compound differed from that published by Hatcher's group (*22*, *31*), who pyrolyzed a similar β-aryl ether model compound and observed large amounts of a product corresponding to 1,2-dimethoxybenzene and a M^+ 208 compound, probably 1-(3,4-dimethoxyphenyl)-2-methoxyprop-1-ene (**10**). MS m/z (%): 208 (M^+, 100), 207 (63), 193 (17), 177 (67), 165 (18), 151 (28). Hatcher's group observed small levels of **1**/**2** and **5**/**6**. The difference in product distribution can be related to TMAH/pyrolysis conditions employed. They employed a so-called off-line TMAH/pyrolysis condition, consisting of a long pyrolysis period and a low temperature (e.g., 300 °C for 10 min (*31*)), while our method consisted of a 4 s pyrolysis period and a 500 °C pyrolysis temperature. As pointed out by Martin et al. (*32*), off-line TMAH/pyrolysis at low temperature may be a different technique from on-line TMAH/pyrolysis at 500 °C.

Our TMAH/pyrograms provided much more structural information on the β-aryl ether subunits as compared to Hatcher's procedure. We observed pyrograms consisting of a larger level of the β-aryl ether subunit-derived fragments and a smaller level of secondary products such as 3,4-dimethoxybenzaldehyde (*33*). Consequently, enol ethers (*Z*/*E*)-**1**/**2** and trimethoxypropanes *erythro*/*threo*-**5**/**6** are proposed as main fragments derived from the guaiacyl β-aryl ether subunits on the basis of the TMAH/pyrolysis product profile of the model compound. Similarly, the 3,4,5-trimethoxybenzene equivalents (**3**/**4** and **7**/**8**) are proposed as main syringyl β-aryl ether subunit-derived fragments (**Figure 2**). Consequently, as indices for the β-aryl ether subunits, the enol ethers (**1**/**2** and **3**/**4**) and trimethoxypropanes (**5**/**6** and **7**/**8**) were chosen in this study, even though the former products do not retain an intact arylglycerol moiety involved in the β-aryl ether subunits. Their MS data are shown in **Table 2**.

TMAH/Pyrolysis−GC Characterization of Lignin. In this study, solid TMAH was used for the in situ lignins at 500 °C, the same temperature as that in TMAH/pyrolysis of *Eucalyptus* woods (*23*), because it provided pyrograms showing methylated products in larger abundances than the TMAH methanolic solution employed for **19** and isolated lignins (*14*). Hardell and Nilvebrant obtained ∼95% of the total TMAH/pyrolysis products from spruce milled wood lignin at 360 °C. However, under our pyrolysis conditions, in situ lignins produced pyrograms with increasing number and abundances of methylated products at higher temperatures. Similar results were reported on humic matters (*32*, *34*) and milled beech leaf litter (*35*). Effects of TMAH/pyrolysis conditions on the profile of the product distribution will be described elsewhere.

Nonmethylated and diazomethane-methylated samples were subjected to the TMAH/pyrolysis analysis; the nonmethylated samples gave the larger pyrolytic S/G ratios. In particular, this trend was more pronounced in the samples with higher syringyl lignin content. In the TMAH/pyrolysis of such samples, the formation of the guaiacyl type products, in particular, the small GC signals of **5**/**6** appearing immediately after the large GC signals of **3**/**4**, made the determination of the signal areas rather difficult. Experimental uncertainties may have led to an underestimation of the contribution of the guaiacyl type products. The increase of the yields of the trimethoxypropanes was required. A solution to this problem is to methylate the sample meal with diazomethane prior to analysis.

TMAH/pyrolysis proceeds under basic conditions. Therefore, the behavior of the β-aryl ether subunits in TMAH/pyrolysis is similar to that during a soda-pulping process. On the basis of model experiments treating β-aryl ether type lignin model compounds with 2 N NaOH at 170 °C for 2 h (*36*, *37*), Gierer (*38*) clarified that in the soda-pulping process phenolic β-aryl ether subunits mainly produce styryl aryl ether subunits and nonphenolic β-aryl ether subunits are extensively cleaved to yield α,β-glycolic subunits (e.g., **19** produced 1-(3,4-dimethoxyphenyl)propane-1,2,3-triol in a 65.1% yield (*37*)). This suggests that in TMAH/pyrolysis protecting phenolic hydroxyl groups of β-aryl ether subunits suppresses the formation of the enol ethers and results in the yield increase of the trimethoxypropanes. As expected, the TMAH/pyrograms of the diazomethane-methylated samples displayed the enol ethers in small amounts and the trimethoxypropanes in large amounts, as compared to those of the nonmethylated samples. This facilitates the determination of the GC signal areas of **5** and **6**, leading to a decrease in experimental uncertainties. The diazomethane-methylated samples provide pyrolytic S/G ratios close to the thioacidolytic S/G ratios.

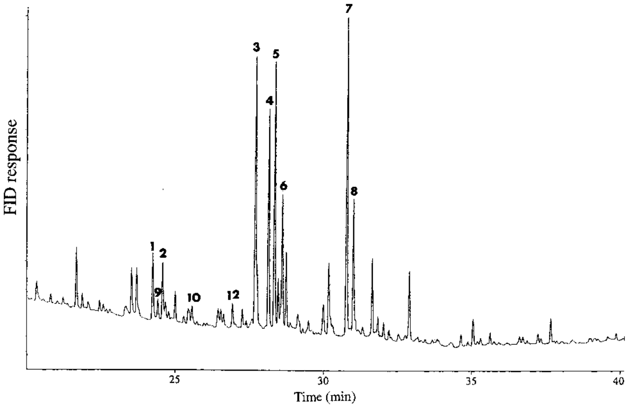

Figure 4. TMAH/pyrogram of diazomethane-methylated beech (sample C). The names and the MS data for compounds **1**–**8** are provided in **Table 2**.

No qualitative differences were observed among the TMAH/pyrograms of samples A−F. **Figure 4** shows the partial TMAH/pyrogram of diazomethane-treated beech (sample C). It is apparent that there are small levels of methylated guaiacyl enol ethers **1**/**2** and large levels of trimethoxypropanes **5**/**6**; this profile of the guaiacyl type products greatly reflects the effect of the diazomethane methylation. Large levels of methylated syringyl equivalents, **3**/**4** and **7**/**8**, are also observed. The syringyl type product amounts are about twice the guaiacyl type product amounts, as expected from the results of nitrobenzene oxidation and thioacidolysis. The signal areas derived from the β-aryl ether subunits contributed about 60% to the summed GC signal areas in the observed GC region (retention time of 20−40 min); this contribution is 1.5 times that (40%) in the spruce milled wood lignin pyrogram obtained by Hardell and Nilvebrant (*20*). Reagent-derived products and most of the subproducts, such as 3,4-dimethoxybenzoic acid methyl ester (*31*) and 3,4-dimethoxybenzaldehyde (*33*), were observed at retention times of <20 min. Small abundances of oxidation products such as 3,4-dimethoxybenzoic acid methyl ester and 3,4-dimethoxybenzaldehyde are noted as compared to other TMAH/pyrolysis pyrograms (*18*, *20*, *31*). Small abundances of dimeric products were observed at retention times of >40 min.

The composition of the β-aryl ether subunits in the lignins in diazomethane-treated meals was obtained from the relative ratio of the sum of GC signal areas of key products observed after TMAH/pyrolysis (**Table 1**). The contribution (relative molar yield %) of each product was calculated by dividing its GC signal area by its relative molar sensitivity; that is, the flame

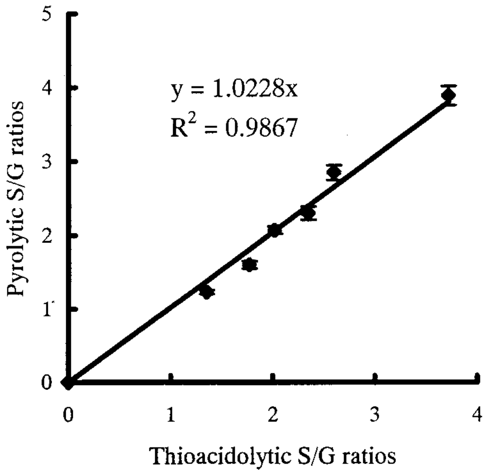

Figure 5. Correlation of the pyrolytic and thioacidolytic S/G ratios. Bars represent standard deviations.

ionization detector response was divided by the effective carbon numbers (*14*, *39*). The total contribution of the key TMAH/pyrolysis products was set at 100%.

The pyrolytic S/G ratio was determined by dividing the summed contribution of the syringyl type products (**3**/**4** + **7**/**8**) with that of the guaiacyl ones (**1**/**2** + **5**/**6**). The data, as seen in **Table 1**, clearly indicate that TMAH/pyrolysis of the diazomethane-methylated in situ lignins provides S/G ratios comparable with the thioacidolytic ones. As shown in **Figure 5**, the pyrolytic and thioacidolytic S/G ratios are well-correlated, with a significant linear regression ($R^2 = 0.9867$).

In addition to the dominant products derived from the β-aryl ether subunits, the severe TMAH/pyrolysis conditions ($pK_a = 9-12$ and 500 °C × 4 s) produced many products. They may come from carbohydrate-derived products, other lignin structures (e.g., 3-(3,4-dimethoxyphenyl)-1-methoxyprop-2-ene (**12**), probably from coniferyl alcohol-end groups), and the subproducts of the β-aryl ether subunits such as **10**. The contributions of these products and the secondary products were not determined. This may produce the errors in the β-aryl ether subunit determination. However, such a treatment does not lead to big determination errors because the secondary products were obtained in small abundances, as shown in **Figure 4**.

Reproducibility of TMAH/Pyrolysis−GC as a Tool for Determining the S/G Ratios. An important indicator of the suitability of an analytical technique for quantitative analysis is its reproducibility. To determine the reliability of the TMAH/pyrolysis method, we obtained RSD values for a minimum of six replicate runs (**Table 1**); the TMAH/pyrolysis−GC runs for one sample were done over 1 week. The RSD (%) was determined by dividing the standard deviation by the mean value. The RSD (2.4%) for sample A shows that the value is comparable with those values of nitrobenzene oxidation and thioacidolysis (3.1 and 1.5%, respectively). The RSD was 3.1% on average for samples A−F. The RSD values ranged from 2.4 to 3.9%, probably depending on the guaiacyl β-aryl ether subunit content. The samples with low guaiacyl lignin unit content appeared to display the lower reproducibility. TMAH/pyrolysis of such samples produced the small GC signal areas of the guaiacyl type TMAH/pyrolysis products, leading to a determination error of the contribution of these products.

In analytical pyrolysis, RSD values of <3% are feasible for replicate analyses (*40*). However, the quantitative reproducibility of Curie-point analysis typically is 5−15% for duplicate analyses (*41*). The reported RSD values are 1.6−5% for the determination of polybutylene terephthalate with eight TMAH/pyrolysis runs under the same pyrolyzer and pyrofoil Ni−Fe system as those employed here (*42*), and a <6% RSD for polyunsaturated fatty acid oil by pyrolysis in the presence of trimethylsulfonium hydroxide (*43*). RSD values of 1.5% (four repeated runs (*12*), and 5.3% (*13*) for 2.2−3.0 pyrolytic ratios of the syringyl lignin units to the guaiacyl lignin units have been reported for an *Eucalyptus camaldulensis* wood by conventional pyrolysis−GC. Rodrigues et al. also reported 2−3% (*44*) and 6.0% (*11*) RSD values for *E. camaldulensis* wood with 2−2.8 and 1.5−2.6 pyrolytic ratios of the syringyl lignin units to the guaiacyl lignin units. For alkyd paint samples, Hickman and Jane obtained long-term RSD values of 5−10% (*45*). When compared to these reported data, the uncertainties (2.4−3.9%) associated with TMAH/pyrolysis in the S/G ratio determinations are within normal operational variation with in situ lignins; therefore, we consider this satisfactory reproducibility (average RSD = 3.1%). The TMAH/pyrolysis results more closely resemble the S/G ratios from thioacidolysis than from nitrobenzene oxidation, as shown in **Table 1**.

Conventional pyrolysis requires many products to determine the lignin composition (e.g., 13 syringyl type products and 13 guaiacyl type products (*12*, *13*)). However, TMAH/pyrolysis requires only 8 products (4 products each for guaiacyl and syringyl β-aryl ether subunits) and provides good reproducibility, comparable with conventional pyrolysis (*12*, *13*).

Our limited results with kenaf and beech in situ lignins suggest that TMAH/pyrolysis can be confidently used as a routine tool for the determination of the proportion of the syringyl and guaiacyl β-aryl ether subunits in lignin. The procedure has a high degree of reproducibility, convenience, and comparability, in particular, with thioacidolysis.

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