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Pyrolysis-trimethylsilylation analysis of lignin: preferential formation of cinnamyl alcohol derivatives

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Abstract

This paper describes a pyrolysis-trimethylsilylation procedure that is a new pyrolysis-derivatization thermochemolysis procedure of lignin. *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was used as a trimethylsilylating reagent. A bulk dehydrogenative polymer of coniferyl alcohol (G-DHP) and a Japanese cedar (*Cryptomeria japonica* D. Don) wood were pyrolyzed in the presence of BSTFA at 500°C for 4 s. The resultant volatile products were analyzed by gas chromatography/mass spectrometry (GC/MS). The G-DHP and the wood produced large abundances of trimethylsilyl (TMS) derivatized coniferyl alcohol (CA-$(TMS)_2$), in which both the phenolic and alcoholic hydroxyl groups are derivatized with TMS. CA-$(TMS)_2$ accounted for about 68% of the TMS derivatized products in the G-DHP pyrolysis, and about 24% of those in the wood pyrolysis. The results indicated that pyrolysis in the presence of trimethylsilylating reagents such as BSTFA is a potentially useful technique for drawing information on the pyrolytic precursors of cinnamyl alcohols (such as cinnamyl alcohol-end groups) from lignin. Pyrolysis-trimethylsilylation occurs in a one-step process and provides the TMS derivatives that are immediately available for the subsequent GC(/MS) analysis. This method is applicable to all lignocellulosic materials.

Keywords: Pyrolysis-gas chromatography/mass spectrometry; Lignin; Trimethylsilyl (TMS) derivatization; *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA); Coniferyl alcohol

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1. Introduction

Lignin is a phenolic random polymer synthesized in plants by dehydrogenative polymerization of cinnamyl alcohols, consisting of an array of differently bonded phenylpropane units. Its large molecular size, complex structure, and close association with other cell wall biopolymers make lignin intractable for analysis. Several techniques, including spectroscopic and wet chemical methods, have been utilized to characterize lignin. However, most of these techniques in general are tedious, time-consuming, and often require sample pretreatment involving the modification of the chemical structures prior to analysis.

Pyrolysis-gas chromatography(/mass spectrometry) (Py-GC(/MS)) has gained wide acceptance as a method of characterizing lignocellulosic materials [1] because of its high sensitivity, a rapid analysis time, and less sample pretreatments. In particular, pyrolysis-derivatization thermochemolysis, that is, pyrolysis in the presence of alkylating reagents such as tetramethylammonium hydroxide (TMAH) developed by Challinor [2–4] has greatly enhanced the potential for pyrolytic analyses of bio- and geopolymers such as lignins and humic acids [5–15].

TMAH/thermochemolysis provides a large quantity of structurally significant compounds that may be eluted chromatographically; this is not the case with conventional pyrolysis. This method, however, involves thermally assisted hydrolysis due to the inherent alkalinity of the reagent. This results in unfavorable cleavage (or damage) of the substructures in polymers to produce structurally non-specific compounds such as veratraldehyde and veratric acid methyl ester in TMAH/thermochemolysis of lignin [5–12,14,19,20]. Furthermore, insufficient alkylation prior to pyrolysis also produces large abundances of dehydration products such as β,3,4-trimethoxyvinylbenzene [5–12,14,16].

The work of Challinor suggests that applications of the different alkylating reagents allow for the further development of pyrolysis-derivatization thermochemolysis. Instead of widely used TMAH, several on-column alkylating reagents such as trimethylsulfonium hydroxide have been applied to the pyrolysis of polymers [2,7,16–18].

Our research efforts have been directed at exploring new reactive pyrolysis-alkylating reagents. Trimethylsilyl (TMS) derivatization was examined. TMS derivatization reagents are very reactive, and TMS derivatives are more sensitive to the flame ionization detector compared to the underivatized compounds. However, literature references contain a few reports on TMS derivatization in analytical pyrolysis [21,22].

Using a sample introduction apparatus for thermally labile samples, a bulk guaiacyl dehydrogenative polymer of coniferyl alcohol (G-DHP) and a Japanese cedar (*Cryptomeria japonica* D. Don) wood were pyrolyzed at 500°C for 4 s in the presence of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), a representative trimethylsilylating reagent. Pyrolysis in the presence of BSTFA, BSTFA/pyrolysis, produced *E*-coniferyl alcohol di-TMS ether, *E*-CA-$(TMS)_2$, in large abundances from lignins.

2. Materials and methods

2.1. Materials

BSTFA was a commercial product (Tokyo Chemical Industry Co. Ltd., Tokyo, Japan). Coniferyl alcohol was purchased from Aldrich. A bulk guaiacyl dehydrogenative polymer of coniferyl alcohol (G-DHP) and a Japanese cedar (*Cryptomeria japonica* D. Don) wood were the same as those used before [23–25].

2.2. Pyrolysis-gas chromatography/mass spectrometry in the presence of BSTFA (BSTFA/Py-GC/MS)

The Py-GC/MS system was a combination of a Curie-point pyrolyzer (JHP-3 model, Japan Analytical Industry Co., Ltd., Tokyo, Japan) with a sample injection apparatus, Bio-Probe (BP-3 model, Japan Analytical Industry Co., Ltd., Tokyo, Japan), and an HP 5890II series gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) with an HP 5972A quadrupole mass detector (Hewlett Packard, Palo Alto, CA, USA). A schematic diagram of the Bio-Probe and pyrolyzer is shown in Fig. 1; detailed description of the Bio-Probe is given in the Oguri's work [26]. The samples (about 100–200 μg) were placed on pyrofoils. BSTFA (3 μl) was dropped on the sample with a microsyringe. The mixture of the sample and the BSTFA was tightly wrapped in the pyrofoil, and the pyrofoil was then inserted to the top (Position A in Fig. 1) of the tube in the dry ice-cooled Bio-Probe, which was mounted in the pyrolyzer heated at 250°C. After the system had been flushed with helium for 15 s, the pyrofoil was dropped into the bottom (Position B in Fig. 1) of the sample tube, where it was pyrolyzed at 500°C for 4 s. The volatile products were sent to the gas chromatograph with a split injection (1:100) via a transfer line heated at 250°C. Helium was used as the carrier gas (flow rate 1.3 ml min^{-1}). The products were separated on a Quadrex fused-silica capillary column (MS, 30 m × 0.25 mm; 0.25 μm film thickness). The temperature program used was 5°C min^{-1} from 50 (1 min) to 300°C, after which isothermal conditions were kept for 9 min. The injection and detector ports were kept at 280°C. All pyrolysis products referred to were ionized by electron impact (70 eV). Spectra were acquired by an Hewlett Packard ChemStation software package. The products were assigned on the basis of mass fragmentation patterns, by comparing the spectra and retention times with those of authentic samples, and by searching Wiley/NIST spectral libraries.

3. Results and discussion

3.1. BSTFA/Py-GC/MS of G-DHP

As a trimethylsilylating reagent, the present study used *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). The trimethylsilylating reagents added to samples

must remain in the samples until the pyrolysis starts. However, the volatility of BSTFA is high compared to TMAH, although BSTFA is less volatile (bp 60°C/35 mmHg) than other trimethylsilylating reagents such as hexamethyldisilazane (bp 125°C) and trimethylchlorosilane (bp 57°C). In our Py-GC/(MS) system, the samples wrapped in pyrofoils are held at 250°C in the hot pyrolyzer for a short time prior to pyrolysis. During this pre-heating period, most of the BSTFA added to the samples decomposes or escapes from the Py-GC/MS system prior to pyrolysis. By using the Bio-Probe, a sample introduction apparatus developed for the pyrolysis of thermal labile samples such as a pork lard, Satoh minimized the escape (or decomposition) of the reagents in pyrolysis of synthetic polymers, and successfully obtained the trimethylsilyl (TMS) derivatized products [21]. The present study also used this apparatus in the BSTFA/Py-GC/MS runs of the G-DHP. Preliminary BSTFA/Py-GC/MS runs of the G-DHP without the Bio-Probe failed to produce the TMS derivatized products.

The Bio-Probe has a coolant reservoir and a permanent magnet that are located close to the top of the sample tube in the Bio-Probe (see Fig. 1). The mixtures of the samples and the BSTFA wrapped in the pyrofoils were inserted to the top

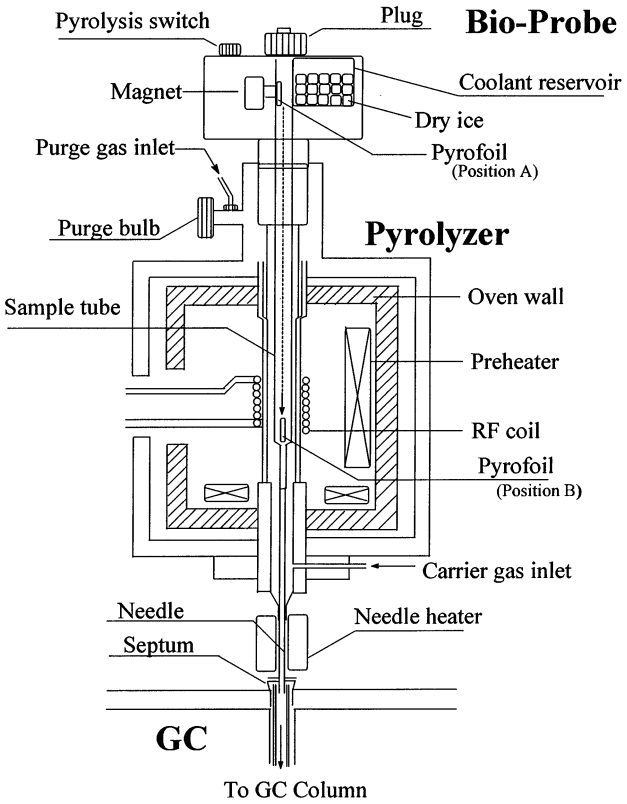

Fig. 1. A schematic diagram of Bio-Probe and pyrolyzer.

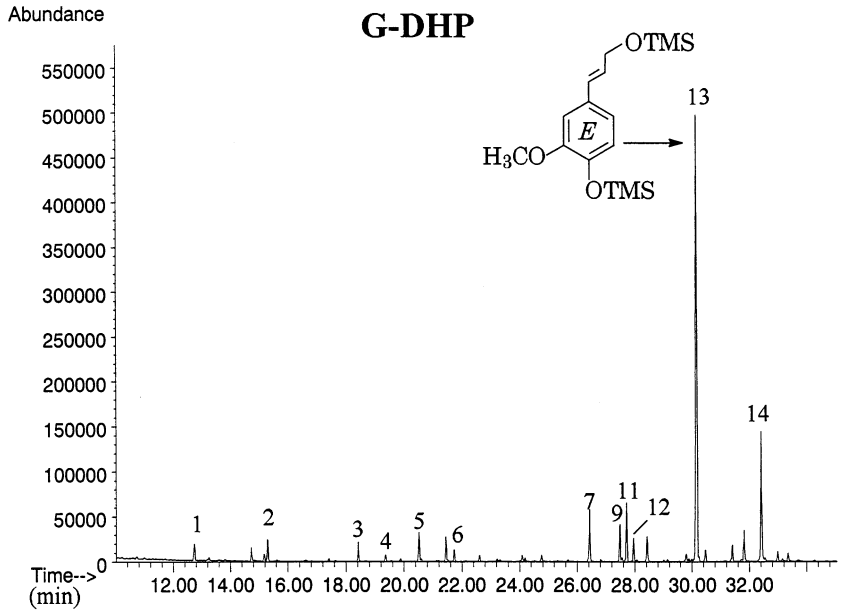

Fig. 2. BSTFA/Py-GC/MS spectrum of G-DHP at 500°C for 4 s. Note: Product numbers and names refer to those in Table 1.

(Position A in Fig. 1) of the tube in the dry ice-cooled Bio-Probe, and the magnet located outside the top of the tube held the pyrofoils. Pyrolysis was triggered as soon as the foil released by the magnet fell to the bottom (Position B in Fig. 1) of the tube. Until the pyrolysis starts, the samples are kept at around 0°C. Therefore, the BSTFA should remain in the pyrofoils until the samples are pyrolyzed.

Fig. 2 shows the Py-GC/MS spectrum of the G-DHP in the presence of BSTFA. Table 1 lists the identified TMS derivatized products with the relative contributions (%). Analytical pyrolysis degrades lignins, which comprise diverse substructures such as the β-aryl ether and β–β′ substructures, to provide a mixture of products retaining the structural attributes of lignins. Therefore, it was anticipated that BSTFA/Py-GC/MS of the G-DHP would produce many TMS derivatives reflecting these substructures. As shown in Fig. 2, however, the profile of product distribution obtained in the BSTFA/pyrolysis of the G-DHP greatly differed from that expected. Only peak 13, retention time of ca. 30 min, was displayed as a main product. This peak was assigned to the *E*-form of coniferyl alcohol di-TMS ether (CA-$(TMS)_2$, M^+ 324), in which both the phenolic and alcoholic hydroxyl groups are derivatized with TMS. This assignment is consistent with the mass spectrum and retention time of the authentic material. Peak 12 at a retention time of 28 min corresponded to *Z*-CA-$(TMS)_2$. The TMS derivatives of vanillic acid (peak 7), coniferaldehyde (peak 9), and dihydroconiferyl alcohol (peak 11) were produced in small abundances. As minor GC/MS peaks, the TMS ethers of guaiacol (peak 1), 4-methylguaiacol (peak 2), and 4-vinylguaiacol (peak 3), eugenol (peak 4), vanillin (peak 5), *E*-isoeugenol (peak 6) were displayed.

Trace abundances of dimeric products were visible above 35 min. This was not a reflection of the sensitivity loss due to the sample amount. Larger sample amounts were pyrolyzed in an attempt to increase the yields of dimeric products. However, the yields of dimeric products did not increase. The reason for the scarcity of the dimeric products is unclear at present. Since no underivatized TMS products were observed, it is believed that the amount of BSTFA is enough to accomplish the pyrolysis-TMS derivatization of the G-DHP. An approximately 15–30:1 weight ratio of BSTFA:sample was used.

To determine the relative contributions of CA-$(TMS)_2$, the summed area of assigned GC/MS peaks (peaks 1–13) was set at 100% and compared to the combined area of the *Z*- and *E*-CA-$(TMS)_2$ peaks (peaks 12 + 13). *Z*/*E*-CA-

Table 1
GC/MS peak assignment in BSTFA/Py-GC/MS of G-DHP and Japanese cedar wood

Peak #	Product	Important MS ion (70 eV), *m*/*z* (%)	Abundance (%)	
			G-DHP	Wood
1	Guaiacol-TMS	196 (M^+, 18.7), 181 (22.9), 166 (100), 151 (23.6), 73 (14.8)	2.5	18.5
2	4-Methylguaiacol-TMS	210 (M^+, 20.1), 180 (100), 73 (10.7)	2.5	12.5
3	4-Vinylguaiacol-TMS	222 (M^+, 25.6), 192 (100), 73 (12.8)	2.1	13.6
4	Eugenol-TMS	236 (M^+, 33.0), 206 (100), 205 (39.1), 73 (37.3)	0.8	2.8
5	Vanillin-TMS	224 (M^+, 25.4), 209 (42.3), 194 (100), 193 (53.9), 73 (24.1)	3.3	2.3
6	*E*-Isoeugenol-TMS	236 (M^+, 38.4), 206 (100), 205 (26.8), 73 (26.0)	1.5	14.3
7	Vanillic acid-$(TMS)_2$	312 (M^+, 58.2), 297 (96.4), 267 (80.3), 253 (54.4), 223 (69.7), 193 (31.1), 126 (41.8), 73 (100)	7.0	Trace
8	Levoglucosan-$(TMS)_3$	378 (M^+, absence), 333 (9.4), 217 (59.7), 204 (67), 73 (100)	–	ND[b]
9	Coniferaldehyde-TMS	250 (M^+,77.5), 235 (32.6), 220 (100), 219 (84.4), 192 (56.5), 73 (70.1)	4.6	Trace
10	*E*-Coniferyl alcohol-TMS[a]	252 (M^+, 74.6), 204 (73.5), 131 (52.2), 73 (100)	–	6.5
11	Dihydroconiferyl alcohol-$(TMS)_2$	326 (M^+, 19.0), 205 (100), 206 (77.4), 179 (33.8), 73 (55.8)	7.3	5.9
12	*Z*-Coniferyl alcohol-$(TMS)_2$	324 (M^+, 52.4), 293 (36.1), 235 (23.1), 204 (33.1), 73 (100)	2.7	3.0
13	*E*-Coniferyl alcohol-$(TMS)_2$	324 (M^+, 52.4), 293 (36.1), 235 (23.1), 204 (33.1), 73 (100)	65.7	20.6
14	Hexadecanoic acid-TMS	328 (M^+, 3.4), 313 (56.0), 145 (28.0), 132 (46.8), 129 (47.0), 117 (100), 75 (74.0), 73 (99.8)	ND	ND
Total			100.0	100.0

[a] Tentatively assigned as the alcoholic hydroxyl group being TMS derivatized.
[b] Not determined.

$(TMS)_2$ accounted for about 68.4% of the assigned products. This shows that BSTFA/pyrolysis selectively cleaved the pyrolytic precursors of coniferyl alcohol (the substructures such as coniferyl alcohol-end groups producing coniferyl alcohol upon pyrolysis [25]) to produce CA-$(TMS)_2$ preferentially. Consequently, the BSTFA/Py-GC/MS spectrum of the G-DHP contained large contributions from the pyrolytic precursors of coniferyl alcohol, and small contributions from the other substructures. The formation of large abundances of Z/E-CA-$(TMS)_2$ in BSTFA/pyrolysis of lignin will facilitate the assessment of the pyrolytic precursors of cinnamyl alcohols.

The product profile of the G-DHP also suggests that in BSTFA/pyrolysis of lignin the substructures with functional groups containing active hydrogen were first derivatized with TMS, followed by subsequent pyrolytic cleavage of the TMS derivatized substructures. The released TMS derivatized products may be subjected to further TMS derivatization.

TMAH/thermochemolysis produces 1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane, which is derived from the guaiacylglycerol moiety of the β-aryl ether substructures. In contrast, BSTFA/pyrolysis of the G-DHP lacks analogous products containing the intact glycerol side chain, such as guaiacylglycerol tetra-TMS ether. This shows that the BSTFA/pyrolysis procedure gives no direct evidence for the existence of the β-aryl ether substructures in lignin. Apparently, BSTFA/pyrolysis induces less cleavage of the β-aryl ether substructures as compared to TMAH/thermochemolysis. This is due to the neutral pH condition of BSTFA/pyrolysis.

3.2. BSTFA/Py-GC/MS of cedar wood

To confirm the findings obtained by BSTFA/pyrolysis of the G-DHP, a Japanese cedar wood lignin having more complicated chemical structures than those of the G-DHP was pyrolyzed in the presence of BSTFA. Fig. 3 shows the BSTFA/Py-GC/MS spectrum of the cedar wood pyrolyzed at 500°C for 4 s. The cedar wood also produced E-CA-$(TMS)_2$ (peak 13) as a main product. Compared to the BSTFA/Py-GC/MS spectrum of the G-DHP, however, the spectrum of the cedar wood showed a more complicated product profile. The TMS ethers of guaiacol, 4-methylguaiacol, 4-vinylguaiacol, and E-isoeugenol (peaks 1, 2, 3 and 6, respectively) are present in large abundances. The TMS ethers of eugenol and vanillin (peaks 4 and 5, respectively) were observed in small abundances. As the contributions of products 1–3 and 6 became larger, the contribution of Z/E-CA-$(TMS)_2$ was reduced from 68.4% in the G-DHP pyrolysis to 23.6% in the wood pyrolysis. Levoglucosan tri-TMS ether (peak 8) was also observed. Peaks without assignment are probably derived from carbohydrates. Like the spectrum of the G-DHP, the cedar wood spectrum displayed no peaks corresponding to the TMS derivatives derived from the guaiacylglycerol moieties of the β-aryl ether substructures.

Coniferyl alcohol mono-TMS ether (CA-TMS, peak 10, M^+ 252, tentatively assigned), of which the alcoholic hydroxyl group was derivatized with TMS, overlapped trace abundances of coniferaldehyde TMS ether (peak 9). Van der Hage

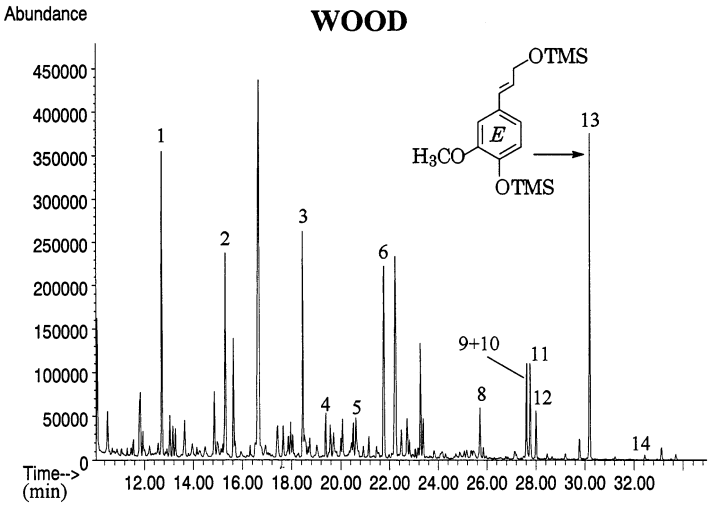

Fig. 3. BSTFA/Py-GC/MS spectrum of Japanese cedar (*C. japonica*) wood at 500°C for 4 s. Note: Product numbers and names refer to those in Table 1.

[22] showed that CA-TMS is derived from coniferyl alcohol-end groups that are β-aryl ether linked via its phenolic hydroxyl group, and CA-$(TMS)_2$ is derived from the guaiacylglycerol moiety of the guaiacylglycerol-β-aryl ether substructures. Clarification of the pyrolytic origins of CA-$(TMS)_2$ and its related compounds in BSTFA/pyrolysis will be the subject of a future study. Future work will also include optimization of the procedure.

4. Conclusions

Pyrolysis-trimethylsilylation has been presented as a means of pyrolysis-derivatization thermochemolysis of lignin. Pyrolysis of lignin with BSTFA produced a large abundance of *E*-coniferyl alcohol di-TMS ether, *E*-CA-$(TMS)_2$, implying that this method induces selective cleavage of the pyrolytic precursors of cinnamyl alcohols. The results therefore indicate that BSTFA/pyrolysis is an effective method for characterizing the pyrolytic precursors of cinnamyl alcohols in lignin. Pyrolysis-trimethylsilylation occurs in a one-step process that provides the TMS derivatives that are immediately available for the subsequent GC(/MS) analysis. This method is applicable to all lignocellulosic materials.

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