Thermochemolytic behavior of β–β lignin structures in the presence of tetramethylammonium hydroxide (TMAH)

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Abstract

Tetramethylammonium hydroxide (TMAH) thermochemolysis is widely used as an effective tool for characterizing hydrolyzable biopolymers such as lignin because it provides more products reflecting structural attributes of the polymer than conventional pyrolysis. However, its functions and the origins of the products are still unclear. Lignin model compounds containing a β–β linkage were subjected to TMAH thermochemolysis (500 °C/4 s) to investigate product distributions and reaction mechanisms. The methylated products were analyzed by gas chromatography/mass spectrometry (GC/MS). Pinoresinols (2,3) and syringaresinol (5) provided di-O-methylpinoresinol (4) and di-O-methylsyringaresinol (6), respectively, as the major product, and methylated monomers. The contribution of di-O-methylresinols was ca 80% in the pyrolyzates based on GC/MS signal areas. A guaiacyl synthetic lignin and a Japanese cedar (Cryptomeria japonica) wood lignin also yielded 4 in large and small abundances, respectively, due to the abundances of β–β subunits in the lignins and the interconnection modes of the β–β subunits with other subunits. The results demonstrated that the TMAH thermochemolysis method is a good tool for analyzing β–β subunits in lignins.

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1. Introduction

Lignin is an important constituent of living, dying and dead biomass, and is also an important precursor of humic acids. It accounts for 15–36% of terrestrial plants (Higuchi, 1985a) and comprises diverse subunits cross-linked by > 10 types of C–C and C–O–C linkages (Adler, 1977). Such complicated chemical structures and close association with other cell wall polymers makes lignin analysis difficult.

There is considerable interest in developing effective methods for the analysis and characterization of lignocellulosic materials and humic substances. Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) has been shown to be a powerful tool owing to its high sensitivity and short analysis times (Chefetz et al., 2002; Hatcher et al., 2001; Meier and Faix, 1992). When pyrolyzed, the lignin polymers are cleaved into smaller structurally representative compounds. The pyrolysis products consist of a wide range of monomeric and oligomeric phenols; however, conventional Py-GC/MS provides less than ideal conditions for the latter products due to poor chromatographic behavior. Therefore, conventional Py-GC/MS of lignocellulosic materials often provides biased pyrograms favoring monomeric products of low polarity.

A pyrolysis method involving methylation with tetramethylammonium hydroxide (TMAH) has emerged as an alternative method to solve this serious analytical problem (Challinor, 1989). Recent studies (Challinor, 1995; Clifford et al., 1995; Filley et al., 1999; Challinor, 2001) showed that this method is a thermally assisted chemolytic reaction, so-called TMAH thermochemolysis. Methylation of acidic functional groups on both
monomeric and oligomeric units decreases their overall polarity, which results in greatly improved chromatography. This extends the applications of TMAH thermochemolysis to the analysis of polymers with multi, polar functional groups (Challinor, 2001) because conventional pyrolysis excludes released polar products from analysis. Therefore, we anticipated that TMAH thermochemolysis would offer pyrograms with more structural information than conventional pyrolysis without TMAH. If so, TMAH thermochemolysis should provide insight into the behavior of lignin at the molecular level, for example the behavior of lignin during fungal decay (Filley et al., 2002; Vane et al., 2001), mineralization (Boyle et al., 1992; Tuomela et al., 2002), solubilization (Boyle et al., 1992), and reaction with pollutants (Brunow et al., 1998, 2002).

We are presently exploring the feasibility of TMAH thermochemolysis as a characterization method for lignin (Kuroda et al., 2001, 2002a–c). For this, a better understanding is required of the behavior of TMAH thermochemolysis of lignin structural subunits. This paper addresses the thermal behavior of lignin subunits of \( \beta-\beta \) type (1) (see Fig. 1 for the chemical structure) in TMAH thermochemolysis. The abundance of \( \beta-\beta \) subunits in lignin structures is said to range from 2% (Adler, 1977) to a high of approximately 17% (Lai and Sarkanen, 1971). Many reports have appeared on the behavior of \( \beta-\beta \) subunits during delignification processes such as pulping (Gierer, 1970, 1985; Gierer et al., 1964; Gierer and Smedman, 1971) and biodegradation by fungi (Higuchi, 1985b). However, available references contain few results on TMAH thermochemolysis of \( \beta-\beta \) subunits, although del Rio et al. (1995) detected di-O-methylsyringaresinol (6) in asphaltenes and kerogens isolated from oil shales.

We pyrolyzed lignin model compounds 2, 3, and 5 containing a \( \beta-\beta \) linkage in the presence of TMAH at 500 °C for 4 s to investigate the TMAH thermochemolysis behavior of \( \beta-\beta \) subunits in lignin. The TMAH thermochemolysis results of reference lignins were compared with those of the model compounds.

2. Materials and methods

Column chromatography (Wakogel C200, Wako Pure Chemical Industries, Osaka, Japan) was carried out with a dichloromethane–methanol (20:1, v/v) solvent system. Thin layer chromatography (TLC) was performed on silica gel plates (Merck, Kieselgel 60 F254, 20 μm thick on aluminum sheet) with the same solvent system as that of column chromatography. Spots were made visible with UV light. \(^1\)H Nuclear magnetic resonance (NMR) spectra were obtained on a JEOL JNM-α400 spectrometer and reported as chemical shifts (relative to tetramethylsilane), splitting patterns, integration areas, and proton assignments. Mass spectrometry (MS) analyses for authentic samples and on-line methylation of the authentic samples with TMAH employed an HP 5890 series II gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) 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) coated with 100% methylsilicone, column temperature 50 °C (1 min), 5 °C min⁻¹ to 300 °C (hold), injector temperature 280 °C, detector temperature 280 °C, helium carrier gas at 30 ml min⁻¹, a jet separator at 175 °C, a source temperature of 200 °C, and an ionization voltage of 70 eV. Spectra were acquired using an HP ChemStation software package. The mass range used was m/z 50–600.

2.1. Materials

Coniferyl alcohol was prepared by reducing acetylferulic acid methyl ester with disobutylaluminum (Aldrich) (Ralph et al., 1992). Sinapyl alcohol was prepared by reducing acetylsinapic acid methyl ester with sodium dihydro-bis(2-methoxyethoxy)aluminate (Wako Pure Chemical Industries) according to the method of Minami et al. (1974). TMAH reagents were purchased from Aldrich.

According to the method of Katayama and Fukuzumi (1978), (+)-pinoresinol (2) was prepared by dehydrogenating coniferyl alcohol enzymatically. A mixture of dehydrogenation products of coniferyl alcohol (5 g) with hydrogen peroxide-berodarshid was chromatographed to produce two (0.6 g) with a TLC pure; mp 157–158 °C (ligroin), lit. mp 156.5–158 °C (Katayama and Fukuzumi, 1978); MS m/z (%) 358 (M⁺, 44), 205 (22), 152 (33), 151 (100), 137 (55), 131 (40).

Di-O-acetylpyrolysinosinol (3) was obtained by acetylation of 2 in a mixture of acetic anhydride and pyridine at room temperature for 12 h; mp 161–162 °C (EtOH/H₂O), lit. (Cooper et al., 1979) mp 153–155 °C. 1H NMR δ (ppm) 2.30 (6H, s, OAc), 3.08 (2H, m, Hβ), 3.83 (6H, s, OMe), 3.92 (2H, dd, J = 9.0, 3.2 Hz, Hγ), 4.27 (2H, dd, J = 9.0, 6.8 Hz, Hγ), 4.78 (2H, d, J = 4.4 Hz, Hα), 6.87 (2H, d, J = 8.1 Hz, Ar-H), 6.97–7.00 (4H, m, Ar-H); the numbering system of α, β, γ is provided in Fig. 1. MS m/z (%) 442 (M⁺, 5), 400 (25), 358 (27), 207 (15), 163 (35), 152 (30), 151 (100), 137 (63).

Di-O-methylpyrolysinosinol (4) was obtained by on-line methylation with TMAH: MS m/z (%) 386 (M⁺, 43), 355 (6), 219 (12), 177 (46), 166 (27), 165 (100), 151 (42).

(±)-Syringaresinol (5) was prepared by dehydrogenating sinapyl alcohol enzymatically, similar to the preparation of 2: mp 172–173 °C (MeOH), lit. mp 170–171 °C (Nimz and Gaber, 1965); MS m/z (%) 418 (M⁺, 100), 182 (32), 181 (89), 167 (70).

Di-O-methylsyringaresinol (6) was obtained by on-line methylation with TMAH: MS m/z (%) 446 (M⁺, 49), 281 (42), 208 (24), 207 (100), 195 (21), 181 (25).

Two reference lignins, a bulk dehydrogenation polymer of coniferyl alcohol (G-DHP) and a Japanese cedar (Cryptomeria japonica) wood lignin, were used as reference lignins; these were the same as those used before (Kuroda et al., 2002b).

G-DHP (230 mg) was treated with a mixture (5 ml) of pyridine and acetic anhydride (1:1, v:v) at room temperature for 12 h. The mixture was poured into cold water (500 ml) and then the precipitate was collected by centrifugation, washed with water, and air-dried to provide an acetylated G-DHP (200 mg).

2.2. 13C NMR spectrometry

13C NMR spectra were obtained on a JEOL JNM-α400 spectrometer (100.4 MHz) and reported as chemical shifts (relative to tetramethylsilane), and integration areas. Acetylated G-DHP (120 mg) was dissolved in acetone-d₆ (0.5 ml) in a 5-mm sample tube. Estimation of the linkage distribution in G-DHP was done by integrating 13C NMR signal intensities attributed to side chain carbons between 70 and 90 ppm (Landucci, 1995).

2.3. On-line methylation with TMAH

On-line methylation of resinols 2, 3 and 5 was performed by introducing the 25% TMAH methanolic solutions containing 2, 3 and 5 into the GC injection port heated at 300 °C. GC/MS analyses of the products employed an HP GC/MSD with a Quadrex MS capillary column (a set of the operating conditions are described above). The products were identified by comparing the retention times and MS fragment patterns of the products with those of standard compounds.

2.4. TMAH thermochemolysis–GC/MS

The pyrolysis-GC/MS system was a combination of a Curie-point pyrolyzer (JHP-3 model, Japan Analytical Industry) with a Shimadzu GC-14A gas chromatograph and a Shimadzu QP-1100EX mass spectrometer (Kyoto, Japan). The model compounds (10–15 μg) were placed on a ferromagnetic pyrofoil, and then the TMAH pentahydrate (~1 mg) was added to the resinols. The mixture was tightly wrapped to ensure contact with the pyrofoil. The sample-loaded pyrofoil was introduced into a quartz tube. The sample holder with the quartz tube was mounted on the pyrolyzer heated at 280 °C. After the system had been flushed with helium for 15 s, the quartz tube was centered in the pyrolyzer and was pyrolyzed at 500 °C for 4 s. The volatile products were sent to the GC with a splitting ratio of 1:50 via the transfer tube heated at 280 °C. Helium was used as the carrier gas (flow rate 30 ml min⁻¹). The products were separated on a stainless steel capillary column (Hitachi, Ultra-ALLOY-(8H)-1, 30 m×0.8 mm, film thickness 2.0 μm). The temperature program used was 5 °C min⁻¹ from 50 (1 min hold) to 300 °C, after which isothermal conditions were kept at 300 °C for 9 min. The injection port was kept at 280 °C. All the pyrolysis products referred to were ionized at 20 eV in an ion chamber heated at
250 °C. The mass range used was \( m/z \) 50–600 with a 1.3 scans s\(^{-1}\) scan speed. The products were identified by comparing MS data with published data (Kuroda, 2002; Kuroda et al., 2002a,b).

2.5. TMAH thermochemolysis–GC

TMAH thermochemolysis–GC of the bulk G-DHP and the cedar wood was performed according to the method described before (Kuroda et al., 2002b). Product identification was performed based on the TMAH thermochemolysis–GC/MS results.

2.6. Conventional Py-GC/MS

Conventional pyrolysis was performed according to a previous paper (Kuroda et al., 1990). GC/MS analyses of the products were the same as those for on-line methylated products (see above). The products were identified by comparing MS data with published data (Ralph and Hatfield, 1991).

3. Results and discussion

3.1. TMAH thermochemolysis of resinsol 2, 3 and 5

(±)-Pinoreisol (2) and its acetate 3 were subjected to TMAH thermochemolysis. The hot, alkaline conditions of the TMAH thermochemolysis method suggest that the behavior of the β–β subunits during TMAH thermochemolysis is akin to that shown for the behavior of lignin structures of the β–β type during alkaline pulping processes (Fig. 2), like the behavior of the lignin structures of the β-aryl ether (Filley et al., 1999) and β-5 types (Kuroda et al., 2002b). Past research efforts on 2 and 3 (Gierer, 1970, 1985; Gierer and Smedman, 1971) showed that the alkaline pulping cleaves phenolic β–β subunits via the formation of a bis-methylene quinone intermediate 7 which produces buta-1,3-diene 8, after elimination of the terminal alcoholate anions as formaldehyde. Subsequent attack of \( \text{OH}^- \) to quinone 7 releases two guaiacyl anions 9 and succinic aldehyde 10; parts of 9 react with the formaldehyde to yield dianhydridemethane 11. On the other hand, nonphenolic β–β subunits are stable against such hot, alkali conditions (Gierer et al., 1964; Gierer and Smedman, 1971). Therefore, we anticipated that TMAH thermochemolysis of 2 and 3 would produce 1,2-dimethoxybenzene (14, methyl ether of 9), and dimers 12 and 13 (permethylated products of 8 and 11, respectively).

However, our TMAH thermochemolysis results on 2 and 3 greatly differed from expectations. Fig. 3 shows the TMAH thermochemolysis–GC/MS trace of 2. TMAH thermochemolysis of 3 also provided a similar product distribution to that of 2 (the pyrogram is not shown). Table 1 lists the identified TMAH thermochemolysis products with their MS data. The TMAH thermochemolysis–GC/MS trace of 2 displays only dimer (signal 4) with the molecular ion at \( m/z \) 386 as the major product, and the monomers in small abundances. The contribution of the \( \text{M}^+ \) 386 signal was 78% for relative integrated GC/MS signal areas between 5 and 60 min retention time; TMAH thermochemolysis–GC/MS trace of 3 also provided 4 in ca 80% contribution. The high TMAH thermochemolysis efficiency of 2 to produce 4 may be due to very short residence times in the pyrolysis chamber. That is, we did not observe the expected dimeric products.

Fig. 2. Behavior of 2 proposed by Gierer and Smedman (1971) during alkaline pulping process.
with the molecular ions at \( m/z \) 326 and 288 (12 and 13, respectively). The M+ 386 signal is consequently attributed to di-O-methylpinoresinol (4), permethylated product of 2, based on the comparison of the MS data and the retention time with those of the on-line TMAH methylated product of 2. The fragment ions at \( m/z \) 177, 165 and 151 are therefore attributed to the Ar–CH–CH = CH2+ (Ar = 3,4-dimethoxyphenyl) species, the acylium cation (Ar–CO+), and the Ar–CH2+ species (Pelter, 1967), respectively.

Monomeric dimethoxybenzenes, 1,2-dimethoxybenzene (14), 1,2-dimethoxy-4-methylbenzene (16), 4-ethyl-1,2-dimethoxybenzene (17), 1,2-dimethoxy-4-vinylbenzene (18), 1,2-dimethoxy-4-(methoxymethyl)benzene (19), 3,4-dimethoxybenzaldehyde (20), 3,4-dimethoxypropenylbenzene (21), 3,4-dimethoxyacetophenone (22) and 3,4-dimethoxypropiophenone (23) are observed in a small abundance. The relatively low abundance of these monomers demonstrates that the opening and cleavage of the tetrahydrofuran ring of 2 occurred to some

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Table 1
Identified mass signals in TMAH thermochemolysis of pinoresinol (2)

<table>
<thead>
<tr>
<th>Signal #</th>
<th>Scan #</th>
<th>Product</th>
<th>Main mass fragments (rel. int. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>1274</td>
<td>1,2-dimethoxybenzene</td>
<td>138 (100), 123 (50), 95 (75)</td>
</tr>
<tr>
<td>15</td>
<td>1476</td>
<td>2-methoxy-4-methylphenol</td>
<td>138 (90), 123 (100), 95 (47), 77 (30)</td>
</tr>
<tr>
<td>16</td>
<td>1638</td>
<td>1,2-dimethoxy-4-methylbenzene</td>
<td>152 (100), 137 (50), 109 (50), 91 (34), 77 (35)</td>
</tr>
<tr>
<td>17</td>
<td>1944</td>
<td>4-ethyl-1,2-dimethoxybenzene</td>
<td>166 (50), 151 (100)</td>
</tr>
<tr>
<td>18</td>
<td>1946</td>
<td>2-methoxy-4-vinylbenzene</td>
<td>164 (100), 149 (48), 121 (25), 103 (36), 91 (64), 77 (52)</td>
</tr>
<tr>
<td>19</td>
<td>2352</td>
<td>1,2-dimethoxy-4-(methoxymethyl)benzene</td>
<td>182 (40), 151 (100)</td>
</tr>
<tr>
<td>20</td>
<td>2422</td>
<td>3,4-dimethoxybenzaldehyde</td>
<td>166 (100), 165 (70)</td>
</tr>
<tr>
<td>21</td>
<td>2520</td>
<td>1,2-dimethoxy-4-prop-1-enylbenzene</td>
<td>178 (100), 163 (45), 151 (26), 107 (47), 103 (34), 91 (50)</td>
</tr>
<tr>
<td>22</td>
<td>2700</td>
<td>1-(3,4-dimethoxyphenyl)ethan-1-one</td>
<td>180 (48), 165 (100)</td>
</tr>
<tr>
<td>23</td>
<td>2988</td>
<td>1-(3,4-dimethoxyphenyl)propan-1-one</td>
<td>194 (25), 165 (100)</td>
</tr>
<tr>
<td>24</td>
<td>3120</td>
<td>unknown</td>
<td>204 (100), 189 (47), 175 (50), 161 (36)</td>
</tr>
<tr>
<td>25</td>
<td>3388</td>
<td>a dehydrogenation product of 26</td>
<td>218 (100), 203 (45), 115 (26)</td>
</tr>
<tr>
<td>26</td>
<td>3472</td>
<td>C10H16O3</td>
<td>220 (8), 165 (100)</td>
</tr>
<tr>
<td>4</td>
<td>6538</td>
<td>Di-O-methylpinoresinol</td>
<td>386 (30), 177 (70), 165 (100), 151 (80)</td>
</tr>
</tbody>
</table>

a Tentatively identified.
b Identified as 1,2-dimethoxy-4-(3-oxabicyclo3.1.0)hex-2-yl)benzene.
methoxypropane isomers, syringyl equivalents to corresponding to 1-(3,4,5-trimethoxyphenyl)-1,2,3-trimethoxypropane (see Fig. 4), and the formation of monomers involved in the opening of the tetrahydrofuran ring is a minor pathway. In summary, in TMAH thermochemolysis of the β-β subunits, alkaline hydrolysis first occurs to cleave the bond between the β-β subunits and the adjacent lignin subunits, and is followed by methylation to produce permethylated resinsols such as 4. Thermal energy literally assists hydrolysis of the bonds and methylation of the dissociated pinoresinol units, and assists the transmission of released 4 into the GC column from the pyrolysis system as TMAH thermochemolysis is designated as a thermally assisted hydrolysis and methylation process (Challinor, 1995; Clifford et al., 1995).

3.2. Conventional Py-GC/MS of 2

For comparison, conventional Py-GC/MS of 2 was performed at 500 °C for 4 s. Fig. 5 reveals 2 and monomers. As the main monomeric products, 2-methoxy-4-methylphenol, 4-hydroxy-3-methoxybenzaldehyde, 4-hydroxy-3-methoxypropiophenone, and dihydroconiferyl alcohol are observed, together with lesser abundances of 2-methoxyphenol, 2-methoxy-4-vinylphenol, isoeugenol, and 4-hydroxy-3-methoxyacetophenone; 5 also provided 3,5-dimethoxy-4-hydroxyphenyl equivalent monomers. Most of the monomers were observed as the corresponding methylated products in Fig. 3. Therefore, the formation of the monomers observed in Fig. 3 may be due to the high temperature pyrolysis. In fact, TMAH thermochemolysis of 2 at 315 °C provided a scarcity of methylated monomers. In Fig. 5, the contribution of 2 was only 8% based on relative integrated GC/MS signal areas between 5 and 60 min retention time, showing the predominance of the formation of the monomeric products due to the opening and cleavage of the tetrahydrofuran ring. These findings show that conventional pyrolysis fragments β-β subunits to a great extent, unlike TMAH thermochemolysis. The monomeric products are often observed in the pyrolyzates of softwood lignins. Therefore, in conventional pyrolysis the pinoresinol type subunit may be a pyrolytic origin of these monomers, in particular 2-methoxy-4-methylphenol, 4-hydroxy-3-methoxybenzaldehyde, 4-hydroxy-3-methoxypropiophenone, and dihydroconiferyl alcohol.
Products with \( M^+ 204 \) and \( M^+ 206 \) are also observed. The MS data of the \( M^+ 204 \) product was the same as that of 24 observed in the TMAH/pyrogram of 2; this product may be a dehydrogenation product of the \( M^+ 206 \) product. The tentatively assigned \( M^+ 206 \) product [MS \( m/z \) 206 (\( M^+ \), 10), 151 (100), 123 (11)] corresponds to 26; it has a 4-hydroxy-3-methoxyphenyl ring instead of the 3,4-dimethoxyphenyl ring of 26.

### 3.3. TMAH thermochemolysis of reference lignins

Guaiacyl synthetic and native lignins with more complicated systems, in comparison with 2 and 3, were subjected to TMAH thermochemolysis. Because synthetic lignins in general contain a large abundance of the \( \beta-\beta \) subunits (Landucci, 1995; Landucci et al., 1998; Terashima et al., 1996; Tollier et al., 1991), the use of them as reference lignins is advantageous to clarify the usefulness of TMAH thermochemolysis as a tool for analyzing \( \beta-\beta \) subunits in lignins. According to the method of Landucci (1995), the frequency of \( \beta-\beta \) linkages in G-DHP was estimated by integrating the \( ^{13}C \) NMR spectrum obtained for acetylated G-DHP (Fig. 6). Intense signals at 72–73 and 86–87 ppm are attributable to the \( \gamma- \) and \( \alpha- \) carbons, respectively, of the \( \beta-\beta \) subunits. Integration of signal areas of the side chain carbons showed that the frequency of \( \beta-\beta \) linkage is ca. 22%; the value is greater than that (17%) of a Landucci’s
DHP (1995), and those of softwood lignins [2% (Adler, 1977) and 14% (Sakakibara, 1980)]. Synthetic lignins essentially consist of only four linkages, \( \alpha-O-4 \), \( \beta-O-4 \), \( \beta-5 \) and \( \beta-\beta \) linkages (Landucci, 1995; Landucci et al., 1998). In other words, DHPs contain resistant linkages such as 5-5 and \( \beta-1 \) linkages in small and/or trace abundances (Landucci, 1995; Landucci et al., 1998; Terashima et al., 1996; Tollier et al., 1991). Therefore, most of the \( \beta-\beta \) subunits of our DHP are connected with other subunits through the phenolic hydroxy group. We expected that the G-DHP would produce a large abundance of 4 by aryl ether cleavage and subsequent methylation in TMAH thermochemolysis.

With G-DHP and Japanese cedar (Cryptomeria japonica), a softwood, we examined whether (1) these guaiacyl lignins, like model compounds 2 and 3, yield 4 in TMAH thermochemolysis, and (2) the pyrograms reflect the differences in abundances of the \( \beta-\beta \) subunits between the lignins.

The TMAH pyrogram of the bulk G-DHP (Fig. 7) was compared with that of the cedar wood (Fig. 8). In the dimeric product region (retention time of > 40 min) of the TMAH pyrogram of G-DHP, 4 is observed in a large abundance, together with isomeric stilbenes 30 stemming from the \( \beta-5 \) subunits (Kuroda et al., 2002b). On the other hand, the TMAH pyrogram of the cedar wood reveals 4 in a rather small abundance. A comparison of the GC signal area of 4 per the lignin weight between the pyrograms showed that the abundance of 4 in the G-DHP pyrogram was \( \sim 7 \) times as large as that in the cedar wood lignin pyrogram. If TMAH thermochemolysis efficiencies, a combination of aryl ether cleavage and methylation, for \( \beta-\beta \) subunits in the wood are similar to those in the DHP, the \( \beta-\beta \) subunit content in the cedar wood lignin is \( \sim 3\% \). Therefore, the pyrograms reflect the differences in \( \beta-\beta \) subunit contents between these lignins. However, if the cedar wood lignin contains \( > 3\% \) \( \beta-\beta \) subunits as reported [e.g., 14% in

Fig. 6. Partial \(^{13}\text{C} \) NMR spectrum of acetylated G-DHP. Designation for the subunits and signal assignments followed those by Landucci (1995).
softwood lignin (Sakakibara, 1980)], an idea based on the β-β subunit content alone appears to provide an insufficient interpretation of the observed large differences in abundance of \( \beta \) between the pyrograms. That is, the paucity of \( \beta \) in the cedar wood pyrogram may not be an exact reflection of the abundance of the β-β subunits present in the cedar wood.

The differences in modes of interconnections between the β-β subunits and other subunits may provide an alternative explanation for the production of \( \beta \). Tera-shima et al. (1999) observed that thioacidolysis of ginkgo lignin containing 10% β-β linkages, estimated by \( ^{13} \)C NMR, provides β-β subunits-derived dimeric products in a rather smaller abundance than expected. Since thioacidolysis mainly cleaves \( \beta-O-4 \) linkages, the dimeric products obtained should stem from subunits connected on both sides by \( \beta-O-4 \) bonds cleavable with thioacidolysis. Based on this, they demonstrated that the β-β subunits in ginkgo native lignin are connected with other subunits by bonds resistant to thioacidolysis at least on one side. That is, most of the β-β subunits in the ginkgo lignin are not connected by β-O-4 linkages, resulting in the production of a small abundance of thioacidolysis dimeric products stemming from the β-β subunits. This may also be the case for a small contribution of 4 in the cedar wood pyrolyzate because TMAH thermochemolysis also cleaves β-O-4 linkages effectively as well as thioacidolysis. Adler (1977), who estimated the presence of 2% β-β subunits in spruce lignin from acidolysis, also pointed out that if pinoresinol subunits were present in lignin in appreciable amounts, one had to assume that major parts of them are linked to adjacent units by biphenyl and diaryl ether bonds resistant to acidolysis. Determination of \( \beta \), and quantitative evaluation of the TMAH thermochemolysis efficiency of 2 to produce 4 are future subjects.

In the monomeric product region of the G-DHP pyrogram (retention time of 10–40 min), 19 and 20 that were observed in the TMAH thermochemolysis–GC/MS trace of 2 are observed. Therefore, the TMAH thermochemolysis monomeric products of the DHP partly stem from the β-β subunits. However, the contributions of the β-β subunits-derived monomers to the pyrolyzate is rather small because 16, the most abundant product in the TMAH thermochemolysis monomeric products of 2, is observed in the DHP pyrogram in rather small abundances. Trimethoxypropane erythro/threo–29 (29e/29t) stemming from the β-aryl ether subunits, and coniferyl alcohol dimethyl ether (28) stemming from coniferyl alcohol-end groups dominate the monomeric product.

![Fig. 7. TMAH thermochemolysis–GC trace of bulk G-DHP. GC analysis employed a Shimadzu GC-17A and a Quadrex MS fused-silica capillary column (Kuroda et al., 2002b). Product names and structures refer to those in Table 1 and Fig. 1, respectively. *3,4-Dimethoxybenzoic acid methyl ester.](image)
region of the DHP pyrogram, while in the cedar wood pyrogram enol ether Z/E–27, and trimethoxypropane 29e/29t dominate the monomeric product region. 3,4-Dimethoxybenzoic acid methyl ester (peak with an asterisk), a sub-product often observed in TMAH thermochemolysis of lignin, is observed in a small abundance in both pyrograms (see Figs. 7 and 8). Consequently, products stemming from β-aryl ether subunits and coniferyl alcohol-end groups dominated the monomeric product region, and the contribution of the β-β subunits-derived monomeric products was small in the monomeric product region.

4. Conclusions

The thermal behavior of lignin subunits of β-β type (1) was studied by TMAH thermochemolysis, using resinol compounds 2, 3 and 5. Di-O-methylresinols such as di-O-methylnorresinol (4) were the main products in TMAH thermochemolysis of the resinols. β-β subunits in guaiacyl synthetic and native lignins, which have more complicated systems, also provided 4 in TMAH thermochemolysis, although the abundances observed were greatly different. The results demonstrate that the TMAH thermochemolysis method is a good tool for analyzing the β-β subunits in lignins.

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