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Dimeric β-Ether Thioacidolysis Products Resulting from Incomplete Ether Cleavage

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Summary

Four β-ether dimers which result from incomplete β-ether cleavage of syringyl-guaiacyl lignins following non-optimized thioacidolysis/desulfurization of plant materials were identified. The compounds were; the guaiacyl-guaiacyl dimer 3-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxy-4-propylphenoxy)propan-1-ol, the guaiacyl-syringyl dimer 2-(2,6-dimethoxy-4-propylphenoxy)-3-(4-hydroxy-3-methoxyphenyl)-propan-1-ol, the syringyl-guaiacyl dimer 3-(3,5-dimethoxy-4-hydroxyphenyl)-2-(2-methoxy-4-propylphenoxy)-propan-1-ol, and the syringyl-syringyl dimer 3-(3,5-dimethoxy-4-hydroxyphenyl)-2-(2,6-dimethoxy-4-propylphenoxy)-propan-1-ol. Synthesis of each dimer, by extending dimeric lignin model β-ether strategies, provided authentication of the structures.

Introduction

Analytical thioacidolysis (Lapierre 1993; Rolando *et al.* 1992) has become an important tool in lignin characterization. Efficient and relatively specific cleavage reactions produce a small array of monomers from lignins that are analyzed by GC or GC-MS (Lapierre *et al.* 1986; Rolando *et al.* 1992). Characterization of dimeric fragments following Raney nickel desulfurization provides additional information on lignin structure (Lapierre 1993; Lapierre *et al.* 1991).

During our applications of this technique to analysing forage samples, biomimetically lignified suspension cultures, and cell wall preparations, we noted the appearance of peaks (Fig. 1) that were not previously identified. Their mass spectra (from GC-MS) were consistent with the β-ether dimers **1a–d** (Fig. 2). Because the appearance of these dimers among plant thioacidolysis products implies incomplete cleavage of β-ether units in lignin, we sought to firmly identify them by synthesizing the authentic compounds and comparing their GC retention times and mass spectra. The NMR data for these compounds are also valuable for identifying reduced (α-CH2) structures in natural and synthetic lignins.

Results and Discussion

If incomplete ether cleavage occurs during thioacidolysis of a syringyl-guaiacyl lignin, four β-ether dimers (**1a–d**, Fig. 2) can result following Raney nickel desulfurization. The γ-OH of unit A (Fig. 2) remains when the β-ether bond is not broken because a β-SEt group is required for its anchimerically assisted displacement; β-1 and β-5 structures, in which the sidechain remains intact, are similarly hydroxylated (Lapierre *et al.* 1991, Lapierre 1993). The entire set of these compounds can be detected by GC or GC-MS (Fig. 1) of a range of plant materials using conditions that appear to be identical to those in the literature (Lapierre *et al.* 1986, 1991; Rolando *et al.* 1992), although we have not carried out our own optimization studies. As in the case of the willow sample in Figure 1, the β-ether dimers can approach the significance of other dimers. The very detection of these units implies that there are significant portions of the lignin polymer that are linked by three successive ether linkages (or two successive units in terminal units); since thioacidolysis will cleave only ether bonds, these dimers could only be released for detection if they in turn were attached by ethers.

The synthesis of each of the four compounds **1a–d** follows traditional β-ether synthetic schemes (Helm and Ralph 1992; Kratzl *et al.* 1959; Landucci *et al.* 1981; Ralph *et al.* 1986) except for the production of α-CH_2 models from their α-OH analogues. For the guaiacyl compounds **1a** and **1b**, the most effective protecting group was the benzyl ether as it can be removed from compounds **5** *via* simple hydrogenolysis. This approach also allows flexibility in the choice of the phenol added to bromide **2**; prior preparation of propylguaicaol is not required and either eugenol or isoeugenol can be added. For the

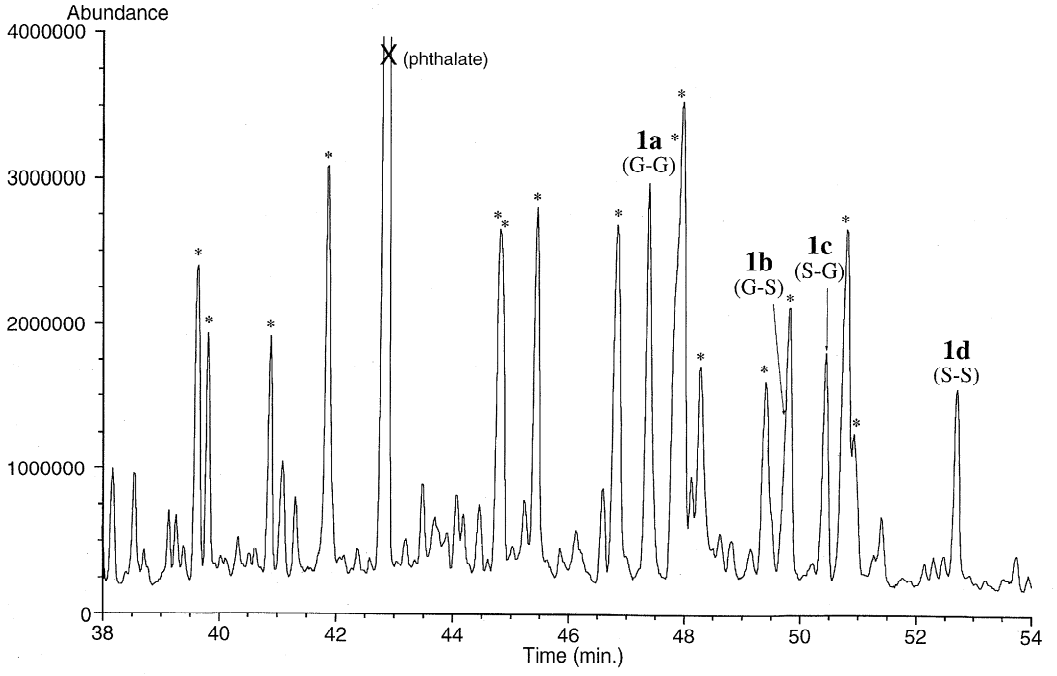

Fig. 1. GC-MS of the thioacidolysis product obtained following thioacidolysis/desulfurization of a willow milled wood lignin. The four β-ether dimers **1a–d** (Fig. 2) discussed in this paper are indicated along with the other non-β-ether dimers (asterisks).

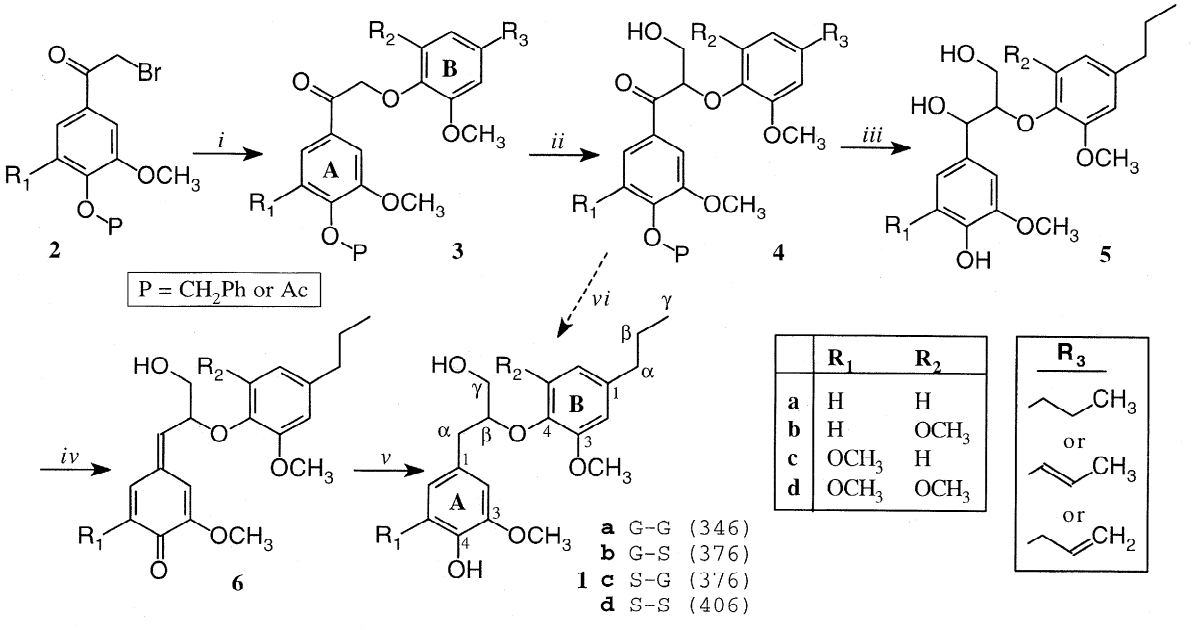

Fig. 2. Synthesis of the four possible dimers **1a–d** that can result from incomplete β-ether cleavage after thioacidolysis/Raney nickel desulfurization of a mixed syringyl-guaiacyl lignin. S = syringyl, G = guaiacyl. The nominal molecular weights (for compounds **1**) are in parentheses. *i* ArOH, K_2CO_3, acetone, reflux (Helm and Ralph 1992; Kratzl *et al.* 1959; Landucci *et al.* 1981; Ralph *et al.* 1986); *ii* $H_2C{=}O$, dioxane, K_2CO_3 (Helm and Ralph 1992); *iii* for P = CH_2Ph, Pd/C–H_2, EtOH (Kratzl *et al.* 1959; Landucci *et al.* 1981; Ralph *et al.* 1986), for P = Ac, $NaBH_4$/EtOH/H_2O (Ralph and Helm 1991); *iv* Me_3SiBr, CH_2Cl_2, followed by $NaHCO_3$ (Ralph and Young 1983); *v* $NaBH_4$ on SiO_2, CH_2Cl_2 (Ralph 1982); *vi* Pd/C–H_2, EtOH+H_2SO_4 (Adler and Marton 1961; Landucci 1980).

syringyl B-ring product **1b**, use of commercially available 4-allyl-2,6-dimethoxyphenol is the simplest approach. However, an acetate protecting group is preferable for syringyl A-ring compounds **1c** and **1d**. Its use requires an additional hydrogenation step to produce the fully saturated sidechain; this can be by making the propylphenol derivatives for the initial reaction with **2** in Figure 2, or by hydrogenation after step (iii) or at the end of the synthesis. The acetate protecting group does however allow its removal

during the borohydride reduction step (iii). In the case of the benzyl-protected compounds **7**, it is possible to effect full reduction to the final products **1** when acidic conditions are used for the Pd-catalysed hydrogenolysis/reduction (Adler and Marton 1961). However, we found the two-step process *via* the quinone methide **6** to be significantly cleaner. The quinone methide reduction by dropping its methylene chloride solution directly onto solid $NaBH_4/SiO_2$ has only been reported previously in a PhD thesis (Ralph 1982), but is a clean, high-yielding straightforward route to the α-CH_2 models from the more commonly prepared α-OH analogues.

Conclusions and Recommendations

Each of the four β-ether dimers **1a–d** (Fig. 2) were located (in variable amounts) in several plant thioacidolysis extracts. In some cases (e.g. Fig. 1), these products of incomplete ether cleavage were quite abundant. This raises interesting basic questions regarding the mechanism and relative cleavage rates of various β-ethers in lignin; clearly, although these dimers contain an intact β-ether bond, neighbouring ethers must be cleaved to release these dimers from the polymer. It should be noted that we did *not* fully optimize the thioacidolysis procedure at our lab, but followed recommendations and conditions for our sample types within the published methods (Lapierre *et al.* 1986, 1991; Rolando *et al.* 1992). The developers of the thioacidolysis method now recommend optimization in each lab, a procedure that can be done within four days. Lapierre (personal communication) states that these products of incomplete ether cleavage rarely result when conditions are carefully optimized. We advise researchers to be aware of these products and suggest that, if the two step thioacidolysis/Raney-Ni desulfurization (Lapierre 1993; Lapierre *et al.* 1991) is run, the appearance of these compounds should be noted so that quantitative data can, if necessary, be adjusted.

Experimental

The four compounds representing all the possible β-ether dimers which would result from incomplete thioacidolysis of a syringyl/guaiacyl lignin were synthesized (Fig. 2) by modifying normal lignin model β-ether strategies (Helm and Ralph 1992; Kratzl *et al.* 1959; Landucci *et al.* 1981; Ralph *et al.* 1986; Ralph and Helm 1991). The synthetic steps up to compounds **5** advance no new methodology and are not described here. The α-CH_2 groups were generated from α-hydroxy parents **5** *via* hydride reduction of their quinone methides (Ralph 1982) as described below. The compounds were individually subjected to GC-MS. Proof of their identity with cell wall thioacidolysis products was obtained by co-injection of plant thioacidolysis products with the synthesized compounds, and by their identical mass spectra.

Thioacidolysis and Raney nickel desulfurizations were performed on 10–20 mg of willow milled wood lignin as described in the originators' references (Lapierre *et al.* 1986, 1991; Rolando *et al.* 1992). The products were silylated with pyridine (15 µl) and BSTFA (30 µl) for 30 min at 60 °C. For mass spectrometry, silylated samples were separated with an HP 5890 gas chromatograph using a 60 m DB-1 capillary column (0.25 mm diam) with He as a carrier gas (0.65 ml/min), and detected with an HP 5970 mass-selective detector. The column was held at 200 °C for 1 min, ramped at 5 °C min^{-1} to 325 °C, and held for 40 min. The injector and detector were set at 300 °C. Synthetic products were treated similarly. High-resolution EI-MS data of synthetic compounds **1a–d** were collected on a Kratos MS-80RFA spectrometer. Percentage values in parentheses refer to the height relative to the spectrum base peak.

NMR spectra of samples in acetone-d_6 were run at 300 K on a Bruker AMX-360 360 MHz narrow-bore instrument fitted with a 5 mm 4-nucleus probe (QNP) with normal probe geometry (proton coil further from sample). Coupling constants are given in Hz. The central solvent signals were used as internal reference (^{1}H, 2.04 ppm; ^{13}C, 29.80 ppm). In some compounds, the coupling to the γ-OH caused γ-protons to be more complex multiplets. In such cases, the chemical shift is taken from the spectrum in acetone, but coupling constants are taken from spectra run after adding 20 µl of D_2O. One and two-dimensional spectra were obtained using standard Bruker pulse programs. Data for compounds **2–5**, as well as complete data for compounds **1** in three solvents (Acetone-d_6, $CDCl_3$, and DMSO), are archived in the recently released NMR Database of Model Compounds for Lignin and Related Plant Cell Wall Components (Ralph *et al.* 1994). Fully authenticated assignments were made using the usual complement of COSY, HMQC and HMBC experiments (Helm and Ralph 1992).

Conversion of compounds **5** to final products **1** *via* quinone methides **6**: α-bromides were prepared in methylene chloride from α-OH compounds **5** (50–100 mg) using TMSBr (2 eq, 15 min. (Ralph and Young 1983). The bromides solution was then shaken with a few ml of saturated aq. $NaHCO_3$, and the yellow methylene-chloride solution of quinone methides **6** was dried over $MgSO_4$ and filtered directly into a flask containing solid $NaBH_4$ on SiO_2 (~5%, Aldrich). After 30 min. when the solution color had changed from yellow to colorless, the solid reductant and support were filtered off and washed with further methylene chloride. The solvent was removed under vacuum to yield crude compounds **1** in *ca* 95% yield. The products **1** were purified by flash chromatography using $CHCl_3$/EtOAc (19 : 1 for **1b**, 3 : 1 for **1c**, 9 : 1 for **1d**) or preparative TLC using 5 : 1 $CHCl_3$: EtOAc as eluant.

3-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxy-4-propylphenoxy)-propan-1-ol **1a** was a colorless oil, ^{1}H NMR δ: 0.89 (3 H, t, J = 7.3, Bγ), 1.58 (2 H, m, Bβ), 2.49 (2 H, m, Bα), 2.91 (1 H, m, Aα_1), 2.94 (1 H, m, Aα_2), 3.59 (2 H, m, Aγ), 3.73 (1 H, t, J = 6.1, Aγ-OH), 3.79 (3 H, s, OMe), 3.80 (3 H, s, OMe), 4.31 (1 H, m, Aβ), 6.65 (1 H, ddt, J = 8.1, 2.1, 0.6, B6), 6.72 (1 H, m, A5), 6.72 (1 H, m, A6), 6.82 (1 H, d, J = 2.1, B2), 6.83 (1 H, d, J = 8.1, B5), 6.92 (1 H, m, A2), 7.33 (1 H, s, Ar–OH); ^{13}C NMR δ: 14.01 (Bγ), 25.39 (Bβ), 37.74 (Aα), 38.22 (Bα), 56.15 (OMe), 56.20 (OMe), 63.38 (Aγ), 83.67 (Aβ), 113.85 (B2), 113.99 (A2), 115.49 (A5), 118.72 (B5), 121.37 (B6), 122.83 (A6), 130.58 (A1), 137.46 (B1), 145.88 (A4), 146.82 (B4), 148.05 (A3), 151.63 (B3); MS m/z 346 (M^+, 27), 181 (27), 180 (13), 166 (52), 151 (11), 138 (43), 137 (100); hi-res MS [Found: M^+, 346.1790, $C_{20}H_{26}O_5$ requires M 346.1780].

2-(2,6-dimethoxy-4-propylphenoxy)-3-(4-hydroxy-3-methoxyphenyl)propan-1-ol **1b** was a colorless oil, ^{1}H NMR δ: 0.92 (3 H, t, J = 7.3, Bγ), 1.62 (2 H, m, Bβ), 2.52 (2 H, dd, Bα), 2.94 (1 H, dd, J = 13.6, 8.2, Aα_1), 3.08 (1 H, dd, J = 13.6, 5.4, Aα_2), 3.39 (1 H, m, J = 12.0, 4.1, Aγ_1), 3.48 (1 H, m, J = 12.0, 3.4, Aγ_2), 3.5 (1 H, m, Aγ-OH), 3.81 (3 H, s, A-OMe), 3.82 (6 H, s, B-OMe's), 4.13

(1H, m, Aβ), 6.54 (2H, s, B2,6), 6.71 (1H, dd, J = 8.0, 1.8, A6), 6.74 (1H, dd, J = 8.0, 0.4, A5), 6.89 (1H, d, J = 1.8, A2), 7.35 (1H, s, Ar–OH); ^{13}C NMR δ: 14.06 (Bγ), 25.30 (Bβ), 38.00 (Aα), 38.91 (Bα), 56.17 (A-OMe), 56.41 (B-OMe's), 62.77 (Aγ), 85.24 (Aβ), 106.55 (B2,6), 113.87 (A2), 115.50 (A5), 122.78 (A6), 130.81 (A1), 134.92 (B4), 139.25 (B1), 145.78 (A4), 148.03 (A3), 154.09 (B3,5); MS m/z 377 (9), 376 (M^+, 33), 197 (25), 196 (91), 195 (20), 181 (27), 167 (75), 151 (10), 137 (100); hi-res MS [Found: M^+, 376.1890, $C_{21}H_{28}O_6$ requires M 376.1886].

3-(3,5-dimethoxy-4-hydroxyphenyl)-2-(2-methoxy-4-propylphenoxy)propan-1-ol **1c** was a colorless oil, ^{1}H NMR δ: 0.89 (3H, t, J = 7.4, Bγ), 1.58 (2H, m, Bβ), 2.49 (2H, m, Bα), 2.85 (1H, dd, J = 13.9, 6.2, $Aα_1$), 2.92 (1H, dd, J = 13.9, 6.1, $Aα_2$), 3.60 (2H, m, Aγ), 3.73 (1H, t, J = 6.1, Aγ-OH), 3.77 (6H, s, A-OMe's), 3.81 (3H, s, B-OMe), 4.33 (1H, m, Aβ), 6.60 (2H, s, A2,6), 6.65 (1H, ddt, J = 8.2, 2.1, 0.6, B6), 6.82 (1H, d, J = 2.1, B2), 6.83 (1H, d, J = 8.2, B5), 6.94 (1H, s, Ar–OH); ^{13}C NMR δ: 14.01 (Bγ), 25.40 (Bβ), 38.21 (Aα), 38.23 (Bα), 56.19 (B-OMe), 56.56 (A-OMe's), 63.46 (Aγ), 83.47 (Aβ), 107.93 (A2,6), 113.81 (B2), 118.54 (B5), 121.36 (B6), 129.59 (A1), 135.34 (A4), 137.38 (B1), 146.85 (B4), 148.48 (A3,5), 151.57 (B3); MS m/z 377 (10), 376 (M^+, 33), 213 (12), 212 (16), 211 (37), 210 (25), 198 (40), 197 (15), 196 (23), 182 (11), 181 (12), 170 (9), 169 (57), 167 (100), 151 (6), 137 (43); hi-res MS [Found: M^+, 376.1880, $C_{21}H_{28}O_6$ requires M 376.1886].

3-(3,5-dimethoxy-4-hydroxyphenyl)-2-(2,6-dimethoxy-4-propylphenoxy)propan-1-ol **1d** was a colorless oil, ^{1}H NMR δ: 0.92 (3H, t, J = 7.4, Bγ), 1.62 (2H, m, Bβ), 2.52 (2H, m, Bα), 2.92 (1H, dd, J = 13.6, 8.1, $Aα_1$), 3.07 (1H, dd, J = 13.6, 5.3, $Aα_2$), 3.40 (1H, m, J = 12.0, 4.1, $Aγ_1$), 3.49 (1H, m, J = 12.0, 3.6, $Aγ_2$), 3.47 (1H, m, Aγ-OH), 3.79 (6H, s, A-OMe's), 3.82 (6H, s, B-OMe's), 4.14 (1H, m, Aβ), 6.54 (2H, s, B2,6), 6.57 (2H, s, A2,6), 6.95 (1H, s, Ar–OH); ^{13}C NMR δ: 14.06 (Bγ), 25.30 (Bβ), 38.38 (Aα), 38.80 (Bα), 56.43 (A-OMe's), 56.58 (B-OMe's), 62.93 (Aγ), 85.20 (Aβ), 106.57 (B2,6), 107.83 (A2,6), 129.69 (A1), 134.94 (B4), 135.24 (A4), 139.10 (B1), 148.44 (A3,5), 154.07 (B3,5) [Note that this compound exhibited hindered rotation phenomena at 300 K in its ^{13}C NMR spectra due to rotational isomers freezing out on the ^{13}C-NMR timescale but not on the ^{1}H NMR timescale. The minor rotamer peaks, where they are resolved, are: 38.50 (α), 38.93 (Bα), 129.86 (A1), 134.97 (B4), 135.28 (A4), 139.26 (B1), 148.49 (A3,5), 148.49 (A5), 154.12 (B3,5)]; MS m/z 406 (M^+, 11), 213 (31), 212 (29), 211 (21), 210 (12), 199 (21), 198 (84), 197 (37), 196 (47), 195 (10), 183 (11), 182 (10), 181 (10), 170 (28), 169 (100), 168 (75), 167 (72), 151 (8); hi-res MS [Found: M^+, 406.1983, $C_{22}H_{30}O_7$ requires M 406.1992].

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