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Isochroman lignin trimers from DFRC-degraded *Pinus taeda*¹

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Abstract

A pair of new aryl isochroman lignin trimers, 7-acetoxy-1-{2-acetoxy-1-[4-(3-acetoxyprop-2-enyl)]-2-methoxyphenoxy}-3-(4-acetoxy-3-methoxyphenyl)-4-acetoxymethyl-6-methoxy-isochromans, along with three related dimers were isolated from DFRC degraded loblolly pine (*Pinus taeda* L.) wood following gel permeation and reversed-phase TLC and HPLC. Their structures were elucidated by means of 2D-NMR, FAB-MS and GC-MS analyses. These two trimers contained a unique β -1 inter-unit linkage with a 6-membered di- α -ether ring and with the original sidechain migrated. The mechanistic plausibility of the lignification pathway, the isolation of expected DFRC degradation dimers and trimers, and the tentative identification of the aryl isochroman structure in pine milled wood lignins by NMR spectroscopy, all suggest that such structures, or their precursors, are present in native lignins (in their unacetylated forms). An unrelated trimer possessing 5-5/ β - β interunit linkages was also isolated and characterized. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: *Pinus taeda* L; Lignin; DFRC-degradation; Structural elucidation; Trimer; Softwood

1. Introduction

Structural details of the complex lignin polymer are still emerging. Only recently were dibenzodioxocins discovered in softwood lignins (Karhunen, Rummakko, Sipilä, Brunow & Kilpeläinen, 1995a,b). They have also now been found in hardwoods, grasses and legumes (Galkin, Ämmälähti, Kilpeläinen, Brunow & Hatakka, 1997 (G. Brunow, 1997, unpublished; J. Ralph, 1997, unpublished)). Degradative methods are valuable in producing low molecular weight compounds from which a picture of the original lignin can emerge. Lignin dimers and trimers which contained most of the major inter-unit linkages have been extensively reported from hydrogenolysis (Sakakibara, 1992) and other methods. However,

knowledge about minor lignin inter-unit linkages is still incomplete, and the β -1-units retain some mysteries.

We recently developed a method to cleave α - and β -ethers in lignin by derivatization followed by reductive cleavage—the ‘DFRC’ method (Lu & Ralph, 1997a,b, 1998a,b). Large-scale degradation of pre-extracted loblolly pine (*Pinus taeda*) sapwood provided, in addition to the monomers, some 16 major dimers in which the major lignin interunitary linkages were represented (Peng, Lu & Ralph, 1998). The dimeric products are analogous to those released by the extended thioacidolysis method (Lapierre, 1993; Rolando, Monties & Lapierre, 1992). This paper presents the structural elucidation of two new β -1 trimers **1** and **2**, and three related dimers **3–5**, along with an unrelated trimer **6**, released from the same large-scale DFRC treatment of pine sapwood (Fig. 1). As far as we know, this is the first report of compounds containing β -1 linkages between two units with the A-unit side-chain shifted to the A₆ position. The importance of these compounds is that they, or their precursors, appear to represent structures present in native lignins. They therefore implicate novel reactions during lignification.

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¹ Part 5 in the series “The DFRC Method for Lignin Analysis”. Previous paper: Peng, J., Lu, F., & Ralph, J. (1998). “Part 4. Lignin Dimers Isolated from DFRC-Degraded Loblolly Pine Wood” *J. Agric. Food Chem.*, 46, 553–560.

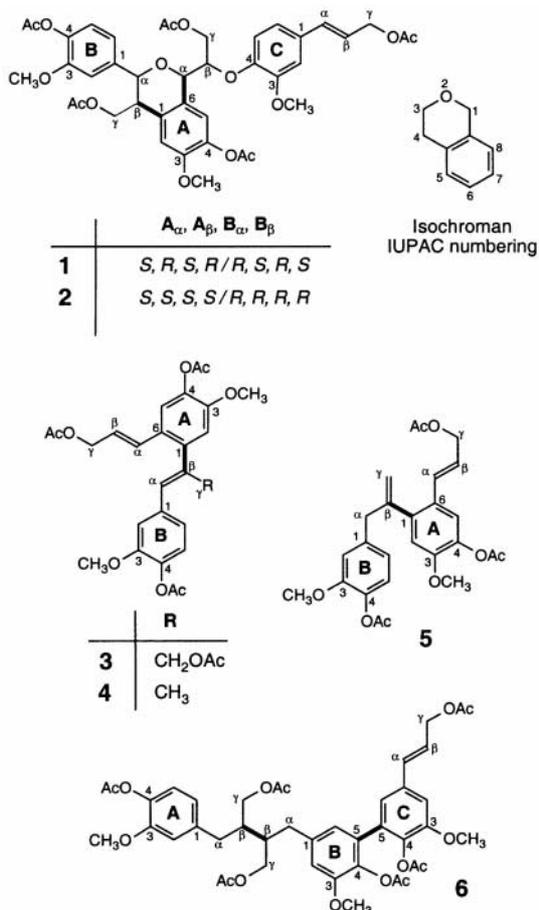


Fig. 1. Structures of new aryl isochroman lignin trimers **1** and **2**, derived dimers **3–5**, and trimer **6**. Numbering uses the standard lignin designations; the isochroman IUPAC numbering is shown to the side of structures **1–2**. Emboldened bonds identify the interunit linkages of interest.

2. Results and discussion

The DFRC-degraded sapwood mixture of loblolly pine (*Pinus taeda* L.) was subjected to gel permeation chromatography on Bio-Beads as described in the previous paper (Peng et al., 1998). The trimer (Fr. 4.3 and Fr. 4.4) and dimer (Bd. 4 and Bd. 6) fractions were further loaded onto the preparative RP-TLC, and finally to preparative-scale HPLC (refer to Fig. 2 in Peng et al., 1998). This led to the separation of new β -1 lignin trimers, isochromans **1** (6.8 mg) and **2** (4.3 mg), along with three related dimers **3–5**, and a 5-5/ β - β trimer **6** (5.7 mg). These compounds were isolated as acetates and were viscous oils.

Compound **1**, an aryl isochroman, was the dominant compound in the trimer fraction. ^1H - and ^{13}C -NMR confirmed **1** as a trimer. Based on HMQC, HMQC-TOCSY, ^1H - ^1H COSY and ^1H - ^1H TOCSY, we unambiguously assigned the protons and carbons for all sidechain and aromatic resonances (see Table 1). As can be seen schematically in Fig. 2, in an HMBC

experiment the long-range correlations of the carbon at δ_{C} 73.8 (B_α) with the proton at δ_{H} 4.75 (1H, *d*, $J = 2.2$ Hz, H- A_α), and a similar correlation for δ_{C} 70.6 (A_α) with δ_{H} 5.37 (1H, *d*, $J = 2.5$ Hz, H- B_α) suggest that there is an α -O- α ether linkage between the A- and B-units. The carbon at δ_{C} 133.0 (A_1) shows correlations with δ_{H} 4.75 (H- A_α), 5.37 (H- B_α), 4.43 (1H, *dd*, $J = 10.9, 4.9$ Hz, H- $B_{\gamma 1}$), and 4.61 (1H, *dd*, $J = 10.9, 8.9$ Hz, H- $B_{\gamma 2}$); δ_{C} 40.4 (B_β) correlates with a singlet proton at δ_{H} 7.20 (1H, *s*, H- A_2). All these correlations support a structure in which carbon B_β links to carbon A_1 , i.e., a β -1 linkage between A- and B-units, and a unique 6-membered ring between the A- and B-unit. Furthermore, δ_{C} 127.3 (A_6) correlates with δ_{H} 4.91 (1H, *ddd*, $J = 5.7, 6.2, 2.2$ Hz, H- A_β), δ_{C} 120.3 (A_5) correlates with δ_{H} 4.75 (H- A_α), and δ_{C} 70.6 (A_α) correlates with δ_{H} 7.13 (1H, *s*, H- A_5) confirming that the sidechain of the A-unit is located at the A_6 position. The carbon at δ_{C} 148.6 (C_4) correlates with δ_{H} 4.91 (H- A_β) confirming that there is a β -O-4 linkage between the A-unit and C-units.

Compound **2** showed similar ^1H and ^{13}C -NMR spectral data to those of **1**. Careful studies on the corresponding correlations on its 2D-NMR spectra convinced us that **2** was an isomer of **1**. Fully authenticated ^1H and ^{13}C -NMR spectral data are listed in Table 1.

2.1. Stereochemistry of **1** and **2**

As shown in Fig. 3, NOESY of **1** showed that H- A_α correlates with H- B_2 , H- B_6 , H- A_5 , H- A_β . H- A_β also sees H- A_5 and H- C_5 , confirming the existence of an α -O- α linkage between A- and B-units, and a β -O-4 linkage between A- and C-units. H- A_2 of **1** correlates with H- B_β and H- B_γ and the methoxyl protons (A_3 -OCH₃). This information further confirmed the linkage of carbon B_β with carbon A_1 , i.e., a β -1 inter-unit linkage between A- and B-units. The data also suggest that the stereochemistry of the preferred α -O- α -ring conformation, defined by the stereochemistry of carbons A_α , A_β , B_α , and B_β to be *SRSR/RSRS* as shown in Fig. 3. Isomer **2** had a correlation between H- A_β and H- B_α in its NOESY spectrum; no such correlation was found in **1**. The coupling constant between H- A_α and H- A_β of **2** (5.2 Hz) is larger than that of **1** (2.2 Hz). Molecular modeling of these two compounds suggested the stereochemistry of carbons A_α , A_β , B_α , and B_β in **2** to be *SSSS/RRRR* as shown in Fig. 3. If it is assumed that the original β -ether unit (defined by moieties **A** and **C**) was ~1:1 *threo:erythro* (*RR/SS:RS/SR*) as is normal in softwoods, the different ring-closing path (**c** \rightarrow **d**, Fig. 4) is logical. Alternative assignments appear to be ruled out by molecular modeling and examination of ^1H - ^1H coupling constants. For example, the *SSSR*-isomer minimizes with $A_\alpha A_\beta$ and

Table 1
 ^1H - and ^{13}C -NMR data for trimers **1** and **2** in acetone- d_6 .

| Position | 1 | | 2 | |
|------------------------|---|-------|---|-------|
| | H | C | H | C |
| A ₄ -Ac Me | 2.17 (3H, <i>s</i>) | 20.4 | 2.17 (3H, <i>s</i>) | 20.5 |
| B ₄ -Ac Me | 2.19 (3H, <i>s</i>) | 20.4 | 2.19 (3H, <i>s</i>) | 20.5 |
| A _γ -Ac Me | 2.01 (3H, <i>s</i>) | 20.8 | 2.01 (3H, <i>s</i>) | 20.8 |
| B _γ -Ac Me | 1.92 (3H, <i>s</i>) | 20.7 | 1.91 (3H, <i>s</i>) | 20.8 |
| C _γ -Ac Me | 2.02 (3H, <i>s</i>) | 20.8 | 2.02 (3H, <i>s</i>) | 20.8 |
| A ₃ -OMe | 3.83 (3H, <i>s</i>) | 56.3 | 3.84 (3H, <i>s</i>) | 56.3 |
| B ₃ -OMe | 3.70 (3H, <i>s</i>) | 56.1 | 3.65 (3H, <i>s</i>) | 56.2 |
| C ₃ -OMe | 3.74 (3H, <i>s</i>) | 56.0 | 3.84 (3H, <i>s</i>) | 56.1 |
| A ₁ | | 133.0 | | 133.3 |
| A ₂ | 7.20 (1H, <i>s</i>) | 113.8 | 7.21 (1H, <i>s</i>) | 113.9 |
| A ₃ | | 151.4 | | 151.5 |
| A ₄ | | 139.9 | | 139.8 |
| A ₅ | 7.13 (1H, <i>s</i>) | 120.3 | 7.06 (1H, <i>s</i>) | 120.5 |
| A ₆ | | 127.3 | | 127.7 |
| A _α | 4.75 (1H, <i>d</i> , 2.2) | 70.6 | 4.77 (1H, <i>d</i> , 5.2) | 70.8 |
| A _β | 4.91 (1H, <i>ddd</i> , 5.7, 6.2, 2.2) | 80.5 | 4.94 (1H, <i>ddd</i> , 6.5, 5.2, 3.7) | 80.4 |
| A _γ | 4.40 (2H, <i>dd</i> , 6.2, 5.7) | 63.6 | 4.34 (1H, <i>dd</i> , 11.7, 3.7), 4.51 (1H, <i>dd</i> , 11.7, 6.5) | 63.5 |
| B ₁ | | 138.5 | | 138.3 |
| B ₂ | 7.06 (1H, <i>d</i> , 1.9) | 113.1 | 7.05 (1H, <i>d</i> , 1.9) | 113.1 |
| B ₃ | | 152.2 | | 152.1 |
| B ₄ | | 140.4 | | 140.5 |
| B ₅ | 6.94 (1H, <i>d</i> , 8.2) | 123.4 | 6.93 (1H, <i>d</i> , 8.2) | 123.4 |
| B ₆ | 6.87 (1H, <i>dd</i> , 8.2, 1.9) | 120.7 | 6.87 (1H, <i>dd</i> , 8.2, 1.9) | 120.9 |
| B _α | 5.37 (1H, <i>d</i> , 2.5) | 73.8 | 5.24 (1H, <i>d</i> , 2.9) | 73.6 |
| B _β | 3.60 (1H, <i>m</i>) | 40.4 | 3.60 (1H, <i>m</i>) | 40.6 |
| B _γ | 4.43 (1H, <i>dd</i> , 10.9, 4.9), 4.61 (1H, <i>dd</i> , 10.9, 8.9) | 66.9 | 4.39 (1H, <i>dd</i> , 10.9, 4.9), 4.48 (1H, <i>dd</i> , 10.9, 8.3) | 66.6 |
| C ₁ | | 132.0 | | 132.3 |
| C ₂ | 7.03 (1H, <i>d</i> , 1.9) | 110.9 | 7.11 (1H, <i>d</i> , 1.9) | 111.3 |
| C ₃ | | 151.6 | | 151.8 |
| C ₄ | | 148.6 | | 148.1 |
| C ₅ | 6.99 (1H, <i>d</i> , 8.3) | 117.5 | 6.98 (1H, <i>d</i> , 8.2) | 118.6 |
| C ₆ | 6.89 (1H, <i>dd</i> , 8.3, 1.9) | 120.4 | 6.89 (1H, <i>dd</i> , 8.2, 1.9) | 120.7 |
| C _α | 6.59 (1H, <i>dt</i> , 15.9, 1.2) | 134.3 | 6.61 (1H, <i>dt</i> , 15.9, 1.1) | 134.2 |
| C _β | 6.22 (1H, <i>dt</i> , 15.9, 6.5) | 123.0 | 6.24 (1H, <i>dt</i> , 15.9, 6.3) | 123.2 |
| C _γ | 4.65 (2H, <i>dd</i> , 6.5, 1.2) | 65.4 | 4.65 (2H, <i>dd</i> , 6.3, 1.1) | 65.4 |
| A ₄ -Ac C=O | | 168.8 | | 168.8 |
| B ₄ -Ac C=O | | 168.9 | | 168.9 |
| A _γ -Ac C=O | | 170.8 | | 170.9 |
| B _γ -Ac C=O | | 171.0 | | 171.0 |
| C _γ -Ac C=O | | 170.8 | | 170.8 |

B_αB_β dihedral angles of ~170 and 180°, inconsistent with the small J_{αβ}'s of 2.2 and 2.5 Hz. X-ray crystallographic confirmation of these assignments will be sought if we can obtain satisfactory crystals.

2.2. Related dimers

We also identified from the dimer fraction three related dimers **3–5** which provide additional proof for the novel structural elements in trimers **1** and **2**. The COSY spectrum of **3** showed that a unique proton at δ_H 6.81 (1H, *br. s*, H-B_α) correlates long-range with protons at δ_H 4.85 (2H, *br. s*, H-B_γ). The NOESY

spectrum showed that the methoxyl protons at δ_H 3.86 (3H, *s*, A₃-OCH₃) correlate with a singlet at δ_H 6.98 (1H, *s*, H-A₂) which correlates with δ_C 135.6 (carbon B_β) in the HMBC experiment. The other singlet at δ_H 7.40 (1H, *s*, H-A₅) correlates with δ_C 130.7 (A_α) and 141.0 (A₁), while the A_β proton at δ_H 6.12 (1H, *dt*, J = 15.9, 6.3 Hz) correlates with δ_C 128.6 (A₆), suggesting that **3** is also a β-1 dimer with the A ring sidechain migrated to carbon-A₆.

As shown in Table 2, ^1H -NMR of **4** also gives two singlets at δ_H 7.36 (1H, *s*, H-A₂), 6.88 (1H, *s*, H-A₅) for ring A and ABX signals in the aromatic region at δ_H 6.49 (1H, *d*, J = 2.0 Hz, H-B₂), 6.83 (1H, *d*,

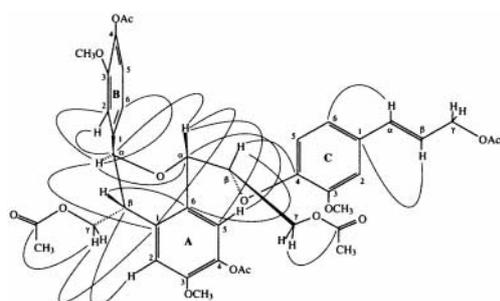


Fig. 2. Major correlations in an HMBC spectrum of trimer **1**.

$J = 8.1$ Hz, H–B₅), 6.64 (1H, *dd*, $J = 8.1$, 2.0 Hz, H–B₆) for ring B. The HMBC experiment showed the methyl protons at δ_{H} 2.15 (3H, *d*, $J = 1.7$ Hz, B₇) correlate with carbons at δ_{C} 128.4 (B₂), 137.7 (B₆) and 140.9 (A₁). In NOESY, the methoxyl protons at δ_{H} 3.80 (3H, *s*, A₃–OCH₃) correlate with a singlet at δ_{H} 6.88 (3H, *s*, H–A₂) which correlates with carbons at δ_{C} 137.7 (B₆) and 127.1 (A₆) in HMBC spectra. Along with the correlation between δ_{H} 6.88 (1H, *dt*, $J = 15.9$, 6.4 Hz, H–A₆) and δ_{C} 127.1 (A₆), the preceding data suggest that **4** is also a β -1 dimer with the sidechain of the A-unit migrated to carbon A₆. W-coupling between H–B₇ and H–B₂ (1.7 Hz) in **4** suggested that the B₂–B₆ double bond was *trans*-substituted by the two aromatic rings, as expected. Both *cis*- and *trans*-isomers of **4** were obtained synthetically to confirm the assignments.

The 2D spectra of **5** possessed analogous patterns to those of **3** and **4**. The methoxyl protons at δ_{H} 3.72 (3H, *s*, A₃–OCH₃) correlate with δ_{H} 6.71 (1H, *s*, H–A₂) which correlates with δ_{H} 3.68 (2H, *br. s*, H–B₂) in NOESY and with δ_{C} 148.6 (carbon B₆) and δ_{C} 127.5 (A₆) in the HMBC experiment. Also in the HMBC, δ_{H}

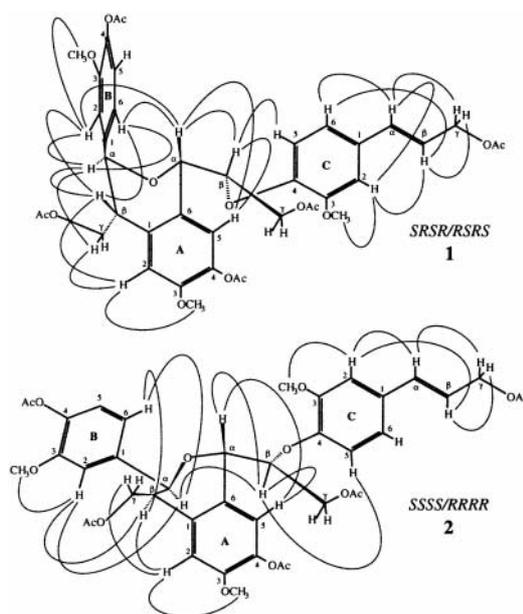


Fig. 3. Major correlations in NOESY spectra of trimers **1** and **2**. Note: the structures are drawn depicting the tentatively assigned stereochemistries although bond lengths and angles have been chosen to allow correlations to be clearly depicted.

7.25 (1H, *s*, H–A₅) correlates with δ_{C} 131.8 (A₂) and 141.0 (A₁), while the A₆ proton at δ_{H} 6.11 (1H, *dt*, $J = 15.9$, 6.3 Hz) correlates with δ_{C} 127.5 (A₆), one of the B₇ protons at δ_{H} 4.95 (1H, *br. d*, $J = 1.8$ Hz, H–B₇) correlates with δ_{C} 141.0 (A₁) and the other at δ_{H} 5.31 (1H, *q*, $J = 1.5$ Hz, H–B₇) correlates with δ_{C} 45.1 (B₂). These data suggest that **5** is also a β -1 dimer with the A-ring sidechain migrated to carbon A₆.

It seems logical that dimers **3**–**5** result from DFRC cleavage of cyclic ether structures represented in

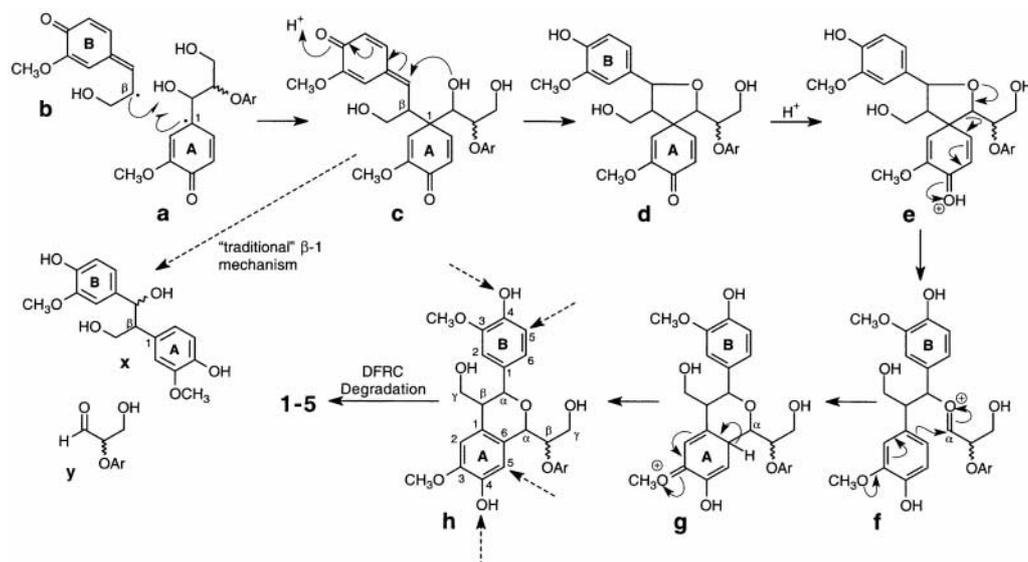


Fig. 4. Proposed lignification/degradation mechanisms of the β -1 inter-unit linkage with the sidechain of A-unit migrated to carbon A₆. (Dashed arrows indicate potential sites of further radical coupling during lignification).

Table 2
 ^1H - and ^{13}C -NMR data for β -1 dimers **3–5** in acetone- d_6 .

| Position | 3 | | 4 | | 5 | |
|------------------------|--------------------------|-------|--------------------------|-------|--------------------------|-------|
| | H | C | H | C | H | C |
| A ₄ -Ac Me | 2.18 (3H, s) | 20.4 | 2.15 (3H, s) | 20.4 | 2.21 (3H, s) | 20.4 |
| B ₄ -Ac Me | 2.25 (3H, s) | 20.5 | 2.24 (3H, s) | 20.5 | 2.24 (3H, s) | 20.5 |
| A _γ -Ac Me | 1.95 (3H, s) | 20.7 | 1.95 (3H, s) | 20.7 | 2.01 (3H, s) | 20.8 |
| B _γ -Ac Me | 2.02 (3H, s) | 20.8 | | | | |
| A ₃ -OMe | 3.86 (3H, s) | 56.3 | 3.80 (3H, s) | 56.5 | 3.72 (3H, s) | 56.2 |
| B ₃ -OMe | 3.39 (3H, s) | 56.1 | 3.37 (3H, s) | 55.8 | 3.70 (3H, s) | 55.3 |
| A ₁ | | 141.0 | | 140.9 | | 141.0 |
| A ₂ | 6.98 (1H, s) | 113.9 | 6.88 (1H, s) | 112.9 | 6.71 (1H, s) | 113.7 |
| A ₃ | | 152.8 | | 152.9 | | 152.7 |
| A ₄ | | 136.7 | | 140.5 | | 140.1 |
| A ₅ | 7.40 (1H, s) | 121.1 | 7.36 (1H, s) | 121.1 | 7.25 (1H, s) | 120.8 |
| A ₆ | | 128.6 | | 127.1 | | 127.5 |
| A _α | 6.69 (1H, dt, 15.9, 1.3) | 130.7 | 6.62 (1H, dt, 15.9, 1.4) | 130.8 | 6.73 (1H, dt, 15.9, 1.3) | 131.8 |
| A _β | 6.12 (1H, dt, 15.9, 6.3) | 124.9 | 6.12 (1H, dt, 15.9, 6.4) | 124.6 | 6.11 (1H, dt, 15.9, 6.3) | 123.9 |
| A _γ | 4.53 (2H, dd, 6.3, 1.3) | 65.2 | 4.54 (1H, dd, 6.4, 1.4) | 65.3 | 4.63 (1H, dd, 6.3, 1.3) | 65.4 |
| B ₁ | | 135.5 | | 136.6 | | 138.7 |
| B ₂ | 6.56 (1H, d, 2.0) | 112.6 | 6.49 (1H, d, 2.0) | 112.1 | 6.81 (1H, d, 2.0) | 114.4 |
| B ₃ | | 152.5 | | 151.6 | | 152.2 |
| B ₄ | | 140.3 | | 139.5 | | 139.4 |
| B ₅ | 6.88 (1H, d, 8.2) | 123.3 | 6.83 (1H, d, 8.1) | 123.1 | 6.91 (1H, d, 8.0) | 123.4 |
| B ₆ | 6.74 (1H, dd, 8.2, 2.0) | 123.1 | 6.64 (1H, dd, 8.1, 2.0) | 122.2 | 6.75 (1H, dd, 8.0, 2.0) | 122.1 |
| B _α | 6.81 (1H, br. s) | 130.4 | 6.58 (1H, q, 1.7) | 128.4 | 3.68 (2H, br. s) | 45.1 |
| B _β | | 135.6 | | 137.7 | | 148.6 |
| B _γ | 4.85 (2H, br. s) | 69.6 | 2.15 (3H, s) | 27.9 | 4.95 (1H, br. d, 1.8) | 117.5 |
| | | | | | 5.31 (1H, q, 1.5) | |
| A ₄ -Ac C=O | | 169.0 | | 168.9 | | 168.9 |
| B ₄ -Ac C=O | | 169.1 | | 169.1 | | 169.0 |
| A _γ -Ac C=O | | 170.6 | | 170.6 | | 170.6 |
| B _γ -Ac C=O | | 170.7 | | 170.7 | | 170.6 |

trimers **1–2**, although isolation of **1** and **2** following DFRC suggests that cleavage was incomplete. Isolation and identification of compounds **1–5** suggest that the interunitary linkage (β -1 with sidechain shifted) shown in compounds **1–5**, or in their precursors (Fig. 4), may exist in the original wood lignin. Model studies are needed to confirm whether compounds **3–5** are from trimers **1–2**, but they are rational products (Peng et al., 1998).

2.3. Proposed mechanism

To date, several dimers or trimers containing β -1 linkages have been isolated or characterized from various degradations of wood, and several possible pathways to these β -1 compounds have been proposed (Nimz, 1965; Lundquist & Miksche, 1965; Sudo & Sakakibara, 1974; Yasuda & Sakakibara, 1976). However, to the best of our knowledge, the cyclic β -1 linkage with a sidechain shifted from carbon A₁ to carbon A₆ has not been reported as such. We suspect that β -6 structures identified by Sakakibara's group (Sudo & Sakakibara, 1974; Yasuda & Sakakibara, 1976, 1977) in hydrogenolysis products are actually analo-

gous sidechain-shifted isomers—we are currently seeking compounds from those authors to clarify this. As for the biogenetic pathway to the cyclic β -1 structures, we propose the pathway shown in Fig. 4. Coupling of a β -O-4 dimer (or higher oligomer) radical **a** and a coniferyl alcohol radical **b** affords intermediate **c** as described previously (Lundquist & Miksche, 1965; Harkin, 1967). Instead of, or in competition with, fragmentation to produce the normal β -1 product **x** and the glyceraldehyde-2-aryl ether **y** (Lundquist & Miksche, 1965; Harkin, 1967), the intramolecular addition of the hydroxyl on the sidechain of the A unit to the B ring quinone methide gives a 5-membered cyclic ether spiro-compound **d**. This is analogous to the internal trapping of quinone methides that occur in formation of β -5 (phenylcoumaran) and β - β (resinol) structures (Harkin, 1967). Protonation to **e** and subsequent rearrangement involves logical migration of the sidechain of the A-unit from carbon A₁ to carbon A₆, forming intermediates **f** and **g**. Note that it is the (original) A-ring sidechain that migrates, not the B-ring sidechain. In such 'dienone-phenol rearrangements' (Miller, 1968), the migrating carbon bears a positive charge; the ether ring oxygen stabilizes the mi-

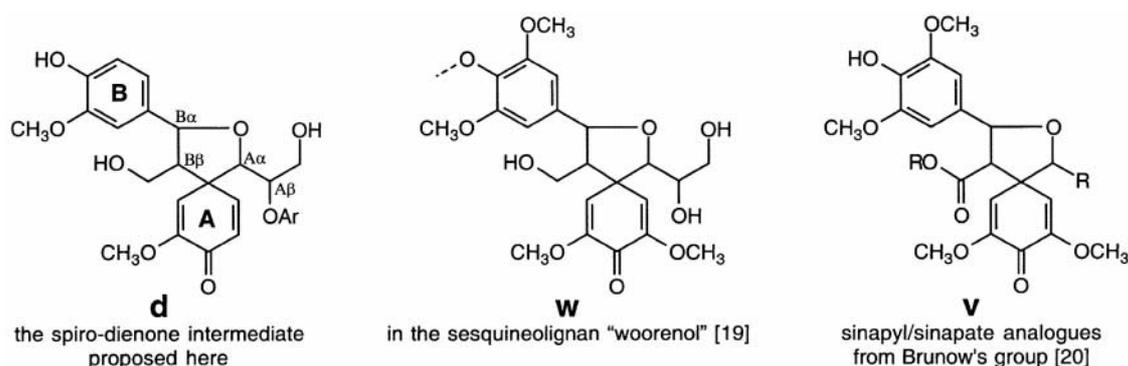


Fig. 5. Comparison of the spiro-dienone intermediate **d** proposed here with the salient structural features of the recently isolated natural product 'wooreno' **w** (Yoshikawa et al., 1997), and sinapyl/sinapate spiro-dienones **v** prepared by radical coupling reactions (Brunow, 1998).

grating positive center at A_α (see structure **f** in Fig. 4). Final aromatization of ring A results in structure **h**. Continued lignification allows radical coupling at the 4-*O*- and 5-sites of rings A and B as shown by dashed arrows in Fig. 4. Compounds **1–5** are produced in the dimeric and trimeric fractions when the resulting lignin fragment **h** undergoes DFRC degradation.

A natural spiro-dienone, wooreno **w** (Yoshikawa, Kinoshita & Arihara, 1997), has recently been reported that contains the important structural features of our intermediate **d** (Fig. 5). Based on NMR data, they assigned their isomer with B_α , B_β as *RS*, as for our compound **1**. Their product was optically active, as may be expected for a sesquieolignan. We shall soon evaluate the optical activity of a range of DFRC products, including trimers **1** and **2**, that retain chiral centers formed in the radical coupling step of lignification. Brunow's group (Brunow, 1998) has prepared, by radical coupling, similar sinapyl/sinapate analogs **v** (Fig. 5).

Tentative support for the occurrence of units containing structures **1–2** in lignin is available from NMR. HMQC or HSQC spectra of some pine lignins show a convincing correlation between carbon B_β ($\delta_C \sim 40.5$) and proton B_β (δ_H 3.60), and weak correlation peaks for the 6-membered di- α -ether ring side-chain are discernible in TOCSY spectra (Ralph, Peng & Lu, 1998). Frankly, these peaks appear too weak to explain how trimers **1–2** are the dominant trimers following DFRC degradation. Two possibilities are that the DFRC method produces these structures from intermediates (e.g., the spiro-dienone **d**, Fig. 4) that have not yet been characterized in lignins, as alluded to by Brunow (Brunow, 1998; Brunow, Ämmälähti, Niemi, Sipilä, Simola et al., 1998), or that these structures are extreme branch-points in lignins and may extract poorly into the MWL fraction (which represents only ~15% of the original lignin).

The other trimer, compound **6**, results from a 5-5-unit in lignin in which one moiety is linked to a β - β -

unit and the other is linked β -*O*-4 (to produce the diagnostic double bond in the reductive elimination step of DFRC). Whether the phenols are etherified is not distinguished by the basic DFRC protocol (Lu & Ralph, 1997a,b), although can be in modified procedures (Lu & Ralph, 1998c). It is therefore not known if the 5-5-units were non-, partially or fully etherified; in the latter case, they could have been involved in dibenzodioxocin structures (Karhunen et al., 1995a,b).

3. Conclusions

The isolation of compounds **1–5** following DFRC-degradation of pine wood adds further support for the occurrence of β -1-coupling during lignification. The structures suggest the existence of a plausible new pathway following the radical coupling step, a pathway which involves intramolecular trapping of the β -1 quinone methide followed by sidechain migration. Compounds assigned as having β -6-linkages have resulted from other degradative procedures (Sudo & Sakakibara, 1974; Yasuda & Sakakibara, 1976, 1977); the aromatic-ring substitution patterns vary from those identified here and should be carefully re-examined in light of the more reasonable mechanism shown in Fig. 4. Products with α -6 linkages can result under acidic conditions (Lapierre, 1993), and it is possible that the dienone-phenol rearrangement (**d** \rightarrow **h**, Fig. 4) is mediated by the acidic conditions of the DFRC procedure. The mechanistic plausibility of the reaction pathway, the isolation of expected degradation dimers **3–5**, and the tentative identification of the aryl isochroman structure in pine milled wood lignins by NMR (Ralph et al., 1998), all suggest that such structures, or their precursors, are present in native lignins. Further efforts to elucidate details of such β -1 pathways during lignification are underway in our laboratory.

4. Experimental

^1H - and ^{13}C -NMR, along with 2D-NMR spectra were taken on a Bruker AMX-360 instrument using standard Bruker pulse programs. NMR assignments were made using the usual complement of 1D and 2D spectra (^1H , ^{13}C , TOCSY, HMQC, HMBC, NOESY). The standard conditions used for all samples were 0.1–10 mg of material in 400 μl of acetone- d_6 , with the central solvent peak as internal reference (δ_{H} 2.04, δ_{C} 29.80). HMBC spectra used a long-range coupling delay of 0.1 s; NOESY spectra were acquired with a 0.8 s mixing time; all other parameters were standard. The conditions for GPC (Bio-beads), prep. RP-TLC (Macherey–Nagel), prep. HPLC (25 cm \times 10 mm I.D.), and GC-MS were as described in the previous paper (Peng et al., 1998).

4.1. Large scale DFRC treatment of ground pine wood

Loblolly pine (*Pinus taeda* L.) sapwood (extracted and milled, 40.42 g) was treated by the DFRC method (Lu & Ralph, 1997b) in 2 equal batches. Normal workup produced 31.56 g of degraded wood products. Obviously, the products were largely polysaccharide-derived. Most major dimeric products have been isolated from the products (Peng et al., 1998). This paper deals with the trimer fraction and some dimer fractions from the same products.

4.2. Separation and purification of degraded lignin trimers and dimers

As shown in Fig. 1 of the previous paper (Peng et al., 1998), eluates from the Bio-beads S-X1 column were combined into 7 fractions based on the peaks in UV chromatograms. Peak #4 (5.06 g) was re-chromatographed on the Bio-beads column and gave 5 fractions (Fr. 4.1–Fr. 4.5). Fr. 4.2 (1.67 g), Fr. 4.3 (0.81 g) and Fr. 4.4 (2.06 g) which were primarily trimers showing the similar profiles by HPLC. Reversed phase TLC plates were used to try to remove the overwhelming carbohydrates and some large dimers. Fr. 4.3 and half of Fr. 4.2 were loaded on to the plates (\sim 220 mg/plate, 1 mm thickness). Plates were developed three times with methanol– H_2O (6:4). Six major bands were viewed on the plates (from top to bottom, Bd. 1–Bd. 6). Combination of the bands on the different plates was made according to their GC-MS and HPLC profiles. Bd. 5 showed 3 major trimers by HPLC. Bd. 5 (156 mg, containing mainly carbohydrates, dissolved in 3 ml of acetonitrile) was purified by HPLC with acetonitrile–water as the mobile phase; Flow rate: 1.6 ml/min; The mobile phase gradient started at 50% acetonitrile– H_2O , holding for 1 min, then increased acetonitrile to 62% over 14 min, then

held for 15 min, and again increased to 100% over 10 min, and held for another 17 min, and finally dropped to 50% over 2 min making the total run 60 min for a single injection (3 mg/60 μl). Fifty one injections afforded **1** (6.8 mg), **2** (4.3 mg), and **6** (5.7 mg). A part of the previous dimer fraction Bd. 4 (40 mg) (Peng et al., 1998) was re-purified by the same HPLC, eluted with 53% acetonitrile– H_2O , and yielded a dimer **3** (2.3 mg); 26.2 mg of Bd. 6 (37.6 mg) gave dimers **4** (1.4 mg) and **5** (1.3 mg).

4.2.1. Trimer 1

7-acetoxy-1-{2-acetoxy-1-[4-(3-acetoxyprop-2-enyl)]-2-methoxyphenoxy}-3-(4-acetoxy-3-methoxyphenyl)-4-acetoxymethyl-6-methoxy-isochroman, tentative stereochemistry as shown in Fig. 3. FAB-MS w. NaCl, LiCl, (m/z) 787 ($\text{M} + \text{Na}$) $^+$, 771 ($\text{M} + \text{Li}$) $^+$, 413, 326. ^1H and ^{13}C -NMR, Table 1.

4.2.2. Trimer 2

Same IUPAC name as **1**, tentative stereochemistry as shown in Fig. 3. FAB-MS (m/z) 787 ($\text{M} + \text{Na}$) $^+$, 413, 326. ^1H and ^{13}C -NMR, Table 1.

4.2.3. Dimer 3

3-(4-acetoxy-3-methoxyphenyl)-2-[4-acetoxy-6-(3-acetoxyprop-2-enyl)-3-methoxy]-prop-2-enyl acetate. GC-MS Rt (min): 21.9; m/z (rel. int.): 526 [M^+] (4), 484 [$(\text{M} - 42)^+$] (8), 466(13), 442(12), 424(11), 406(12), 382(17), 363(49), 347(43), 322(100), 291(44), 277(47), 165(28), 137(97). ^1H and ^{13}C -NMR, Table 2.

4.2.4. Dimer 4

3-(4-acetoxy-3-methoxyphenyl)-2-[4-acetoxy-6-(3-acetoxyprop-2-enyl)-3-methoxy]-prop-2-ene. GC-MS Rt (min): 18.5; m/z (rel. int.): 426 [$\text{M} - 42$] $^+$ (26), 384(6), 366(4), 351(2), 324(100). ^1H and ^{13}C -NMR, Table 2.

4.2.5. Dimer 5

3-(4-acetoxy-3-methoxyphenyl)-2-[4-acetoxy-6-(3-acetoxyprop-2-enyl)-3-methoxy]-prop-1-ene. GC-MS Rt (min): 19.6; m/z (rel. int.): 468 [M] $^+$ (0.4), 426 [$\text{M} - 42$] $^+$ (28), 408(0.7), 384(12), 366(7), 351(2), 324(100), 291(17), 187(15), 137(19). ^1H and ^{13}C -NMR, Table 2.

4.2.6. Trimer 6

5'-[4-acetoxymethyl-3-(4-acetoxy-3-methoxybenzyl)-2-acetoxymethyl-butyl]-5-(3-acetoxypropenyl)-2,2'-diacetoxy-3,3'-dimethoxy-biphenyl. FAB-MS (m/z) 815 ($\text{M} + \text{Na}$) $^+$, 551, 413, 326. ^1H NMR: δ 2.13 (3H, s, $\text{A}_4\text{-OAc}$), 2.20 (3H, s, $\text{B}_4\text{-OAc}$), 2.08 (3H, s, $\text{C}_4\text{-OAc}$), 2.01 (3H, s, $\text{A}_7\text{-OAc}$), 2.00 (3H, s, $\text{B}_7\text{-OAc}$), 1.99 (3H, s, $\text{C}_7\text{-OAc}$), 3.75 (3H, s, $\text{A}_3\text{-}$

OCH₃), 3.78 (3H, *s*, B₃-OCH₃), 3.88 (3H, *s*, C₃-OCH₃), 6.91 (1H, *d*, *J* = 1.9 Hz, H-A₂), 6.92 (1H, *d*, *J* = 7.9 Hz, H-A₅), 6.70 (1H, *dd*, *J* = 7.9, 1.9 Hz, H-A₆), 2.68 (1H, *dd*, *J* = 12.2, 8.8 Hz, H-A_{α1}), 2.88 (1H, *dd*, *J* = 12.2, 6.2 Hz, H-A_{α2}), 2.26 (1H, *m*, H-A_β), 4.06 (1H, *dd*, *J* = 11.4, 5.4 Hz, H-A_{γ1}), 4.25 (1H, *dd*, *J* = 11.4, 6.3 Hz, H-A_{γ2}), 6.92 (1H, *d*, *J* = 1.9 Hz, H-B₂), 6.67 (1H, *d*, *J* = 1.9 Hz, H-B₆), 2.75 (1H, *dd*, *J* = 12.2, 8.9 Hz, H-B_{α1}), 2.89 (1H, *dd*, *J* = 12.2, 6.3 Hz, H-B_{α2}), 2.26 (1H, *m*, H-B_β), 4.02 (1H, *dd*, *J* = 11.4, 5.4 Hz, H-B_{γ1}), 4.22 (1H, *dd*, *J* = 11.4, 6.3 Hz, H-B_{γ2}), 7.25 (1H, *d*, *J* = 2.0 Hz, H-C₂), 6.86 (1H, *d*, *J* = 2.0 Hz, H-C₆), 6.69 (1H, *dt*, *J* = 15.9, 1.4 Hz, H-C_α), 6.38 (1H, *dt*, *J* = 15.9, 6.3 Hz, H-C_β), 4.70 (2H, *dd*, *J* = 6.3, 1.4 Hz, H-C_γ). ¹³C NMR: δ 20.3 (A₄-OAc), 20.3 (B₄-OAc), 20.5 (C₄-OAc), 20.8 (A_γ-OAc), 20.8 (B_γ-OAc), 20.8 (C_γ-OAc), 56.5 (A₃-OCH₃), 56.4 (B₃-OCH₃), 56.1 (C₃-OCH₃), 136.9 (A₁), 114.1 (A₂), 152.3 (A₃), 139.2 (A₄), 123.4 (A₅), 121.6 (A₆), 35.5 (A_α), 40.9 (A_β), 64.7 (A_γ), 131.9 (B₁), 113.5 (B₂), 152.1 (B₃), 139.4 (B₄), 138.5 (B₅), 123.7 (B₆), 35.5 (B_α), 41.2 (B_β), 64.7 (B_γ), 135.5 (C₁), 110.4 (C₂), 152.7 (C₃), 140.1 (C₄), 132.7 (C₅), 121.8 (C₆), 133.4 (C_α), 125.3 (C_β), 65.1 (C_γ), 168.6 (A₄-OAc), 168.8 (B₄-OAc), 169.1 (C₄-OAc), 170.8 (A_γ-OAc), 171.1 (B_γ-OAc), 170.8 (C_γ-OAc).

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