The DFRC Method for Lignin Analysis. 7. Behavior of Cinnamyl End Groups

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The behavior of cinnamyl end groups of lignins during the derivatization followed by reductive cleavage (DFRC) procedure has been investigated using lignin model compounds. On AcBr treatment, hydroxycinnamyl alcohols give rise mainly to 1-aryl-1,3-dibromopropanes from which 1-aryl-3-bromopropanes and arylcyclopropanes are formed by zinc reduction. Arylpropene derivatives are also significant among DFRC products of etherified cinnamyl end-group models. Major monomers from DFRC of hydroxycinnamaldehydes are arylcyclopropyl acetates produced by reductive ring closure of 1-acetoxy-3-aryl-1,3-dibromopropanes. Although the reactions are not as clean as the ether-cleaving reactions that form the basis of the DFRC method, end groups produce diagnostic compounds that provide valuable markers for studying end groups in lignins.

Keywords: *Acetyl bromide; lignin model compound; end groups; β-bromoether; reductive elimination; DFRC; hydroxycinnamyl alcohol; hydroxycinnamaldehyde; arylcyclopropane*

INTRODUCTION

Lignins are complex natural polymers arising from an enzyme-mediated dehydrogenative polymerization of phenylpropanoid precursors, primarily coniferyl and sinapyl alcohols (Harkin, 1967; Freudenberg and Neish, 1968; Adler, 1977; Chen, 1991). Softwood guaiacyl lignins are derived primarily from coniferyl alcohol, whereas hardwood guaiacyl—syringyl lignins come from a mixture of coniferyl and sinapyl alcohols. Lignification involves free-radical coupling reactions, sometimes combined with nucleophilic additions to quinone methide intermediates, to form three-dimensional polymers. Sarkanen (1971) reviewed the two types of polymerization processes for synthetic lignin (DHP) formation in vitro originated by Bernd Lehman and John M. Harkin (Harkin, personal communication). The Zutropf DHPs, formed by adding lignin precursors slowly and continuously, were called "endwise" polymers and structurally resembled isolated wood lignins more closely than Zulauf DHPs or "bulk" polymers, formed by adding the precursors in a single batch.

One characteristic difference between endwise and bulk synthetic lignin polymers is that there are fewer cinnamyl end groups in the former than in the latter, because bulk lignification involves substantial immediate dimerization. Endwise polymerization more frequently involves addition of a monomer to a growing lignin oligomer. Because the number of cinnamyl end groups in lignin is relatively low, lignification in the plant cell wall is believed to be an endwise polymerization, although there is considerable evidence for cytochemical heterogeneity in lignins (Grushnikov and Shorygina, 1970; Terashima et al., 1979, 1986a,b; Lapierre and Monties, 1981, 1991; Monties and Lapierre, 1981; Lapierre et al., 1982, 1983, 1991; Monties, 1985; Tollier et al., 1986; Terashima and Fukushima, 1988; Terashima and Seguchi, 1988; He and Terashima, 1990, 1991; Fukushima and Terashima, 1991; Kim et al., 1994; Ede et al., 1996). Recently we found that a milled tobacco lignin, like DHPs, has a high content of cinnamyl end groups, β—5 and β—β linkages (Ralph et al., 1998). The content of end groups in lignins is therefore an important characteristic of lignin structure. It would be helpful to be able to quantify lignins' end groups for a better understanding of lignin biosynthesis.

The DFRC (**d**erivatization **f**ollowed by **r**eductive **c**leavage) method is a recently developed analytical tool for lignin characterization (Lu and Ralph, 1997a,b, 1998a—c; Peng et al., 1998, 1999; Ralph and Lu, 1998). Through DFRC, β-aryl ether linkages in lignin are cleaved, releasing monomers that are quantified by GC. Most monomeric and dimeric DFRC products have been identified. A previous study (Lu and Ralph, 1998a) showed that monomers released from end groups were diagnostic, but a mechanism to account for their formation was not clear. In this study several lignin models with cinnamyl end groups were subjected to DFRC degradation and major monomers isolated and identified. Mechanisms leading to the formation of these diagnostic monomers are addressed.

EXPERIMENTAL PROCEDURES

^{1}H, ^{13}C, and 2D ^{1}H—^{13}C correlation NMR (gradient HMQC and HMBC) spectra were taken on a Bruker DRX-360 instrument fitted with a 5-mm probe with inverse geometry (proton coils nearest the sample) and three-axis gradients. The conditions used for all samples were 2—60 mg of material in 0.4 mL of acetone-d_6, with the central solvent peak as internal reference (δ_H 2.04, δ_C 29.80). The carbon/proton designations are based on the conventional lignin numbering system (Figures 3 and 6).

GC and GC/MS conditions were as described previously (Lu and Ralph, 1998a). Mass spectra for brominated products are reported by specifying the bromine-derived multiplets followed

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by their relative intensities, e.g. 322/324/326 (4/8/4)—the triplet signifying an ion with two bromines.

The DFRC Procedure. For GC/MS analysis, 5−10 mg of substrates was used for DFRC. For preparative scale DFRC, 100−150 mg of starting materials was used.

AcBr treatment conditions used were those previously described (Lu and Ralph, 1997b).

AcBr treatment products were separated on normal-phase preparative (2-mm thickness) TLC plates (Alltech, Deerfield, IL) using $CHCl_3$/EtOAc (20:1) as solvent. The major DFRC final products **11**, **14**, and **23** were isolated from C_{18} reverse-phase 1-mm TLC plates (Alltech) using MeOH/water, 6:4, following normal-phase TLC ($CHCl_3$/EtOAc, 20:1) from preparative DFRC of 4-hydroxycinnamyl alcohols **1** and 4-hydroxycinnamaldehydes **15**.

Product Characterization. Structures for compounds in this section are in Figures 2 and 3. Mass spectra for the final monomeric products were reported in part 2 of this series (Lu and Ralph, 1998a).

*3-Acetoxy-1-(4-acetoxy-3-methoxyphenyl)-1-bromopropane (**3a**):* δ_H 1.97 (γ-OAc), 2.23 (A-OAc), 2.56 (2H, m, β), 3.85 (OMe), 4.14 (1H, m, γ), 5.29 (1H, dd, J = 8.7, 6.4 Hz, α), 7.04 (1H, d, J = 8.0 Hz, A5), 7.08 (1H, dd, J = 8.0, 2.0 Hz, A6), 7.25 (1H, d, J = 2.0 Hz, A2); δ_C 20.4 (γ-OAc), 20.7 (A-OAc), 39.3 (β), 52.3 (β), 56.3 (OMe), 62.8 (α), 112.7 (A2), 120.4 (A6), 123.8 (A5), 141.0 (A4), 141.4 (A1), 152.4 (A3), 168.9 (A-OAc), 170.8 (γ-OAc); MS 344/346 (M^+ 2/2), 302/304 (3/3), 243/245 (1/1), 223 (5), 205 (15), 163 (92), 150 (25), 131 (100).

*1-(4-Acetoxy-3-methoxyphenyl)-1,3-dibromopropane (**4a**):* δ_H 2.23 (OAc), 2.62 (1H, m, β1), 2.83 (1H, m, β2), 3.57 (2H, m, γ's), 3.85 (OMe), 5.31 (1H, dd, J = 9.0, 5.8 Hz, α), 7.05 (1H, d, J = 8.1 Hz, A5), 7.10 (1H, dd, J = 8.1, 2.0 Hz, A6), 7.26 (1H, d, J = 2.0 Hz, A2); δ_C 20.4 (OAc), 31.9 (γ), 42.7 (β), 53.7 (α), 56.4 (OMe), 112.7 (A2), 120.4 (A6), 123.9 (A5), 140.7 (A1), 141.1 (A4), 152.4 (A3), 168.9 (A-OAc).

*1-(4-Acetoxy-3,5-dimethoxyphenyl)-1,3-dibromopropane (**4b**):* δ_H 2.23 (OAc), 2.62 (1H, m, β1), 2.83 (1H, m, β2), 3.57 (2H, m, γ's), 3.83 (6H, s, OMe's), 5.28 (1H, dd, J = 9.0. 5.8, α), 6.90 (2H, s, A2/6); δ_C 20.2 (OAc), 31.9 (γ), 42.6 (β), 54.2 (α), 56.6 (OMe's), 105.1 (A2/6), 129.8 (A4), 140.1 (A1), 153.2 (A3/5), 168.4 (A-OAc); MS 364/366/368 (M^+, 1/2/1), 322/324/326 (4/8/4), 243/245 (100/98), 164 (24), 150 (12), 149 (11), 137 (35), 129 (33), 103 (19), 91 (20), 77 (17).

*4-Acetoxy-3-methoxycinnamyl bromide (**5a**):* δ_H 2.23 (OAc), 3.83 (OMe), 4.24 (1H, dd, J = 7.8, 0.9 Hz, γ), 6.49 (1H, dt, J = 15.5, 7.8 Hz, β), 6.72 (1H, d, J = 15.5 Hz, α), 7.01 (1H, m, A5), 7.01 (1H, m, A6), 7.20 (1H, s, A2); δ_C 20.4 (OAc), 34.3 (g), 56.2 (OMe), 111.2 (A2), 120.1 (A6), 123.7 (A5), 126.5 (β), 134.5 (α), 135.7 (A1), 140.9 (A4), 152.3 (A3), 168.8 (OAc); MS 284/286 (M^+, 3/3), 242/244 (5/5), 163 (70), 131 (100), 103 (33).

*4-Acetoxy-3,5-dimethoxycinnamyl bromide (**5b**):* δ_H 2.23 (OAc), 3.82 (OMe), 4.25 (2H, dd, J = 7.6. 0.8 Hz, γ), 6.53 (1H, dt, J = 15.5, 7.8 Hz, β), 6.70 (1H, d, J = 15.5 Hz, α), 6.84 (2H, s, A2/6); δ_C 20.2 (OAc), 34.3 (γ), 56.4 (OMe), 104.3 (A2/6), 126.7 (β), 129.9 (A4), 135.0 (α), 135.1 (A1), 153.3 (A3), 168.4 (OAc).

*1-Acetoxy-1-(4-acetoxy-3-methoxyphenyl)-2,3-dibromopropane (**6a**):* δ_H 2.12 (α-OAc), 2.23 (A-OAc), 3.80 (1H, m, γ1), 3.85 (OMe), 3.92 (1H, m, γ2), 4.80 (1H, dt, J = 6.1, 5.9 Hz, β), 6.10 (1H, d, J = 6.1 Hz, α), 7.08 (1H, m, A5), 7.08 (1H, m, A6), 7.25 (1H, s, A2); δ_C 20.5 (α-OAc), 20.8 (A-OAc), 34.7 (γ), 55.2 (β), 56.3 (OMe), 75.8 (α), 112.8 (A2), 120.7 (A6), 123.5 (A5), 136.0 (A1), 141.2 (A4), 152.2 (A3), 168.8 (A-OAc), 169.6 (α-OAc); MS 422/424/426 (M^+, 2/4/2), 380/382/384 (6/12/6), 221 (20), 195 (11), 179 (32), 153 (100), 131 (12).

*1-(4-Acetoxy-3-methoxyphenyl)-1,2,3-tribromopropane (**7a**):* not isolated; structure from GC/MS only; MS 400/402/404/406 ($M^+ - CH_2C{=}O$, 4/6/6/3), 321/323/325 (36/70/37), 242/244 (6/6), 241/243 (4/4), 163 (100), 131 (100), 103 (38).

*1-(4-Acetoxy-3-methoxyphenyl)-3-bromopropane (**11a**):* δ_H 2.16 (2H, m, bs), 2.75 (2H, m, α's), 3.49 (2H, t, J = 6.6 Hz, γ's), 3.80 (3H, s, OMe), 6.80 (1H, dd, J = 8.0, 1.8 Hz, A6), 6.95 (1H, d, J = 8.0 Hz, A5), 6.80 (1H, d, J = 1.80 Hz, A2); δ_C 34.0 (γ), 34.5 (α), 35.1 (β), 56.1 (OMe), 113.6 (A2), 121.1 (A6), 123.4 (A5), 139.2 (A4), 140.5 (A1), 152.1 (A3), 169.1 (A-OAc).

*1-(4-Acetoxy-3,5-dimethoxyphenyl)-3-bromopropane (**11b**):* δ_H 2.16 (2H, m, bs), 2.74 (2H, m, α's), 3.50 (2H, t, J = 6.6 Hz, γ's), 3.80 (6H, s, OMe), 6.80 (2H, s, A2/6); δ_C 34.1 (γ), 35.1 (β), 35.2 (α), 56.4 (OMe's), 106.0 (A2/6), 128.1 (A4), 140.1 (A1), 153.1 (A3/5), 168.6 (A-OAc).

*4-Acetoxy-3-methoxyphenylcyclopropane (**14a**):* δ_H 0.68 (2H, m, β/γ), 0.94 (2H, m, β/γ), 1.90 (1H, m, α), 2.19 (3H, s, A-OAc), 3.76 (3H, s, OMe), 6.66 (1H, dd, J = 8.0, 1.8 Hz, A6), 6.801 (1H, d, J = 1.80 Hz, A2), 6.87 (1H, d, J = 8.0 Hz, A5); δ_C 9.5 (β/γ), 15.9 (α), 20.5 (A-OAc), 56.0 (OMe), 110.9 (A2), 118.0 (A6), 123.2 (A5), 138.7 (A4), 143.7 (A1), 152.1 (A3), 169.0 (A-OAc).

*4-Acetoxy-3,5-dimethoxyphenylcyclopropane (**14b**):* δ_H 0.70 (2H, m, β/γ), 0.93 (2H, m, β/γ), 1.89 (1H, m, α), 2.19 (3H, s, A-OAc), 3.76 (6H, s, OMe's), 6.4 (2H, s, A2/6); δ_C 9.5 (β/γ), 16.5 (α), 20.3 (A-OAc), 56.4 (OMe's), 103.1 (A2/6), 127.7 (A4), 143.6 (A1), 153.0 (A3/5), 168.6 (A-OAc).

*1-(4-Acetoxy-3-methoxyphenyl)-1-bromo-3,3-diacetoxypropane (**17a**):* δ_H 1.99 (3H, s, γ-OAc), 2.00 (3H, s, γ-OAc), 2.22 (3H, s, A-OAc), 2.75 (2H, m, bs), 3.82 (3H, s, OMe), 5.27 (1H, dd, J = 8.2, 7.0 Hz, α), 6.85 (1H, dd, J = 6.6, 4.7 Hz, γ), 7.04 (1H, d, J = 8.1, 2.0 Hz, A5), 7.09 (1H, dd, J = 8.1, 2.0 Hz, A6), 7.27 (1H, d, J = 2.0 Hz, A2); δ_C 20.4 (A-OAc), 20.5 (γ-OAc), 20.6 (γ-OAc), 43.4 (β), 49.08 (α), 56.4 (OMe), 89.5 (γ), 112.6 (A2), 120.4 (A6), 123.8 (A5), 140.9 (A1), 141.1 (A4), 152.4 (A3), 168.9 (A-OAc), 169.0 (γ-OAc), 169.2 (γ-OAc).

*1-(4-Acetoxy-3,5-dimethoxyphenyl)-1-bromo-3,3-diacetoxypropane (**17b**):* δ_H 2.04 (3H, s, γ-OAc_1), 2.05 (3H, s, γ-OAc_2), 2.26 (3H, s, A-OAc), 2.80 (2H, m, β's), 3.86 (6H, s, OMe's), 5.28 (1H, dd, J = 8.2, 6.8 Hz, α), 6.90 (1H, dd, J = 6.6, 4.7 Hz, γ), 6.94 (2H, s, A2/6); δ_C 20.2 (A-OAc), 20.6 (γ-OAc's), 43.4 (β), 49.6 (α), 56.7 (OMe's), 89.6 (γ), 105.2 (A2/6), 129.8 (A4), 140.3 (A1), 153.3 (A3/5), 168.4 (A-OAc), 169.0 (γ-OAc), 169.2 (γ-OAc).

*1-Acetoxy-3-(4-acetoxy-3-methoxyphenyl)-1,3-dibromopropane (**18a**) (two isomers):* δ_H 2.05/2.11 (3H, s, γ-OAc's), 2.23/2.23 (6H, A-OAc), 3.01/3.14 (2H, m, β's), 3.84/3.85 (OMe's), 5.28/5.30 (1H, m, α), 6.63 (1H, dd, J = 6.6, 6.0 Hz, γ), 6.75 (1H, dd, J = 8.5, 4.1 Hz, γ's), 7.11/7.11 (1H, dd, J = 8.2, 2.0 Hz, A6's), 7.05/7.06 (1H, d's, J = 8.0 Hz, A5's), 7.30 (1H, d, J = 2.0 Hz, A2's); δ_C 20.4/20.4 (A-OAc), 20.6/20.7 (γ-OAc), 48.5/49.1 (β), 50.5/50.9 (α), 56.4/56.4 (OMe), 74.0/75.5 (γ), 112.7/112.8 (A2), 120.5/120.5 (A6), 123.9/124.0 (A5), 139.9/140.2 (A1), 141.2/141.2 (A4), 152.4/152.5 (A3), 168.5/168.9 (γ-OAc), 168.8/168.9 (A-OAc).

*1-Acetoxy-3-(4-acetoxy-3,5-dimethoxyphenyl)-1,3-dibromopropane (**18b**) (two isomers):* δ_H 2.06/2.12 (3H, s, γ-OAc's), 2.22/2.22 (6H, s, A-OAc's), 3.15/3.16 (2H, m, bs), 3.82/3.82 (6H, s, OMe's), 5.27/5.29 (1H, m, α), 6.63 (1H, dd, J = 6.1, 6.0 Hz, γ), 6.76 (1H, dd, J = 8.74, 4.0 Hz, γ's), 6.93/6.94 (2H, s, A2/6); δ_C 20.2/20.2 (A-OAc), 20.6/20.7 (γ-OAc), 49.0/48.5 (β), 51.4/51.0 (α), 56.6/56.6 (OMe's), 73.9/75.5 (γ), 105.2/105.2 (A2/6), 129.9/129.9 (A4), 139.6/139.3 (A1), 153.2/153.3 (A3/5), 168.2/168.2 (γ-OAc), 168.3/168.5 (A-OAc).

*4-Acetoxy-3-methoxy-9-bromocinnamyl acetate (**19a**):* δ_H 2.07 (3H, s, γ-OAc), 2.23 (3H, A-OAc), 3.82 (3H, s, OMe), 6.33 (1H, dd, J = 16.1, 6.1 Hz, β), 6.88 (1H, d, J = 16.1 Hz, α), 7.04 (1H, d, J = 8.2 Hz, A5), 7.10 (1H, dd, J = 8.2, 2.1 Hz, A6), 7.25 (1H, dd, J = 6.1, 1.0 Hz, γ), 7.27 (1H, d, J = 2.1 Hz, A2); δ_C 20.4 (A-OAc), 20.7 (γ-OAc), 56.3 (OMe), 90.17 (γ), 111.6 (A2), 120.6 (A6), 123.4 (β), 123.9 (A5), 135.2 (A1), 135.2 (α), 141.5 (A4), 152.6 (A3), 168.8 (A-OAc), 169.1 (γ-OAc).

*1-(4-Acetoxy-3-methoxyphenyl)-1,3,3-tribromopropane (**21a**):* δ_H 2.23 (OAc), 3.17 (1H, m, β1), 3.40 (1H, m, β2), 3.80 (OMe), 5.26 (1H, dd, J = 8.9, 5.5 Hz, α), 5.90 (1H, dd, J = 8.2, 5.5 Hz, γ), 7.07 (1H, d, J = 8.2 Hz, A5), 7.15 (1H, dd, J = 8.2, 2.0 Hz, A6), 7.35 (1H, d, J = 2.0 Hz, A2); δ_C 20.4 (OAc), 43.7 (γ), 52.6 (β), 54.0 (α), 56.4 (OMe), 113.0 (A2), 120.7 (A6), 124.0 (A5), 139.5 (A1), 141.4 (A4), 152.6 (A3), 168.8 (OAc).

*1-(4-Acetoxy-3,5-dimethoxyphenyl)-1,3,3-tribromopropane (**21b**):* δ_H 2.22 (OAc), 3.17 (1H, m, β1), 3.42 (1H, m, β2), 3.84 (6H, s, OMe's), 5.24 (1H, dd, J = 9.1, 5.4 Hz, α), 5.90 (1H, dd, J = 8.3, 5.4 Hz, γ), 6.98 (2H, s, A-2/6); δ_C 20.2 (OAc), 43.8 (γ), 53.2 (β), 53.9 (α), 56.7 (OMe's), 105.5 (A2/6), 130.2 (A4), 138.9 (A1), 153.4 (A3/5), 168.4 (OAc).

*cis-1-Acetoxy-2-(4-acetoxy-3-methoxyphenyl)cyclopropane (cis-**23a**):* δ_H 1.23 (1H, m, β1), 1.30 (1H, m, β2), 1.78 (3H, s, γ-OAc),

2.20 (3H, s, A-OAc), 2.26 (1H, m, α), 3.79 (3H, s, OMe), 4.28 (1H, m, γ), 6.78 (1H, dd, J = 8.0, 1.8 Hz, A6), 6.91 (1H, d, J = 8.0 Hz, A5), 6.92 (1H, d, J = 1.8 Hz, A2); δ_C 11.4 (β), 20.5 (A-OAc), 20.5 (γ-OAc), 22.3 (α), 53.8 (γ), 56.1 (OMe), 113.9 (A2), 121.3 (A6), 122.9 (A5), 136.6 (A1), 139.3 (A4), 151.7 (A3), 169.0 (A-OAc), 171.4 (γ-OAc).

*trans-1-Acetoxy-2-(4-acetoxy-3-methoxyphenyl)cyclopropane (trans-**23a**):* δ_H 1.22 (1H, m, β1), 1.30 (1H, m, 2), 1.98 (3H, s, γ-OAc), 2.20 (3H, s, A-OAc), 2.20 (1H, m, α), 3.80 (3H, s, OMe), 4.14 (1H, m, γ), 6.73 (1H, dd, J = 8.0, 1.8 Hz, A6), 6.90 (1H, d, J = 8.0 Hz, A5), 6.92 (1H, d, J = 1.8 Hz, A2); δ_C 14.9 (β), 20.4 (A-OAc), 20.7 (γ-OAc), 23.6 (α), 56.1 (OMe), 56.5 (γ), 111.8 (A2), 119.1 (A6), 123.4 (A5), 139.3 (A4), 139.9 (A1), 152.2 (A3), 169.0 (A-OAc), 171.5 (γ-OAc).

*cis-1-Acetoxy-2-(4-acetoxy-3,5-dimethoxyphenyl)cyclopropane (cis-**23b**):* δ_H 1.28 (2H, m, β's), 1.80 (3H, s, γ-OAc), 2.19 (3H, s, A-OAc), 2.25 (1H, m, α), 3.77 (6H, s, OMe's), 4.28 (1H, m, γ), 6.56 (2H, s, A2/6); δ_C 11.5 (β), 20.2 (A-OAc), 20.6 (γ-OAc), 22.8 (α), 53.9 (γ), 56.4 (OMe's), 106.2 (A2/6), 128.3 (A4), 136.2 (A1), 152.7 (A3/5), 168.6 (A-OAc), 171.4 (γ-OAc).

*trans-1-Acetoxy-2-(4-acetoxy-3,5-dimethoxyphenyl)cyclopropane (trans-**23b**):* δ_H 1.27 (2H, m, β's), 2.00 (3H, s, γ-OAc), 2.19 (3H, s, A-OAc), 2.20 (1H, m, α), 3.77 (6H, s, OMe's), 4.17 (1H, m, γ), 6.53 (2H, s, A2/6); δ_C 15.0 (β), 20.2 (A-OAc), 20.7 (γ-OAc), 24.1 (α), 56.4 (OMe's), 56.6 (γ), 104.1 (A2/6), 128.2 (A4), 139.5 (A1), 153.1 (A3/5), 168.6 (A-OAc), 171.5 (γ-OAc).

Synthesis of β-Aryl Ether Lignin Models (Figure 5). Etherified end-group lignin models **30** and **31** (Figure 6) were synthesized according to published methods (Ralph and Young, 1981; Kulkarni and Sebastian, 1990; Helm and Ralph, 1992; Lu and Ralph, 1998d).

*β-Coniferaldehyde ether of guaiacyl glycol [1-(4-hydroxy-3-methoxyphenyl)-2-[2-methoxy-4-(prop-2-enal)phenoxy]ethanol (**30a**)]:* δ_H 3.89 (3H, s, B-OMe), 3.93 (3H, s, A-OMe), 4.15 (2H, m, Aβ), 4.55 (1H, d, J = 4.1 Hz, Aα-OH), 5.05 (1H, m, Aα), 6.71 (1H, dd, J = 15.8, 7.8 Hz, Bβ), 6.84 (1H, d, J = 8.4 Hz, A5), 6.97 (1H, dd, J = 8.4, 1.8 Hz, A6), 7.09 (1H, d, J = 8.4 Hz, B5), 7.18 (1H, d, J = 1.8 Hz, A2), 7.27 (1H, dd, J = 8.4, 2.1 Hz, B6), 7.38 (1H, d, J = 2.1 Hz, B2), 7.60 (1H, d, J = 15.8 Hz, Bα), 9.68 (1H, d, J = 7.6 Hz, Bγ); δ_C 56.2 (A-OMe), 56.4 (B-OMe), 72.5 (Aα), 75.6 (Aβ), 111.0 (A2), 111.9 (B2), 114.3 (B5), 115.4 (A5), 119.9 (A6), 124.3 (B6), 127.6 (Bβ), 128.5 (B1), 134.0 (A1), 146.9 (A4), 148.1 (A3), 150.9 (B3), 152.4 (B4), 153.6 (Bα), 193.9 (Bγ).

*β-Sinapaldehyde ether of guaiacyl glycol [1-(4-hydroxy-3-methoxyphenyl)-2-[2,6-dimethoxy-4-(prop-2-enal)phenoxy]ethanol (**30b**)]:* δ_H 3.78 (1H, m, Aβ1), 3.82 (3H, s, A-OMe), 3.93 (6H, s, B-OMe's), 4.28 (1H, m, Aβ2), 4.31 (1H, d, J = 2.4 Hz, Aα-OH), 4.84 (1H, dt, J = 8.7, 2.6 Hz, Aα), 6.76 (1H, dd, J = 15.9, 7.6 Hz, Bβ), 6.77 (1H, d, J = 8.1 Hz, A5), 6.84 (1H, dd, J = 8.1, 1.8 Hz, A6), 7.03 (1H, d, J = 1.8 Hz, A2), 7.11 (2H, s, B2/6), 7.60 (1H, d, J = 15.9 Hz, Bα), 9.67 (1H, d, J = 7.6 Hz, Bγ); δ_C 56.2 (A-OMe), 56.7 (B-OMe's), 72.9 (Aα), 80.5 (Aβ), 107.0 (B2/6), 110.8 (A2), 115.4 (A5), 119.9 (A6), 129.1 (Bβ), 131.1 (B1), 132.9 (A1), 140.5 (B4), 146.9 (A4), 148.1 (A3), 153.4 (Bα), 154.4 (B3/5), 193.9 (Bγ).

*β-(Coniferyl alcohol) ether of guaiacyl glycol [1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropenyl)-2-methoxyphenoxy]-ethanol (**31a**)]:* δ_H 3.83 (3H, s, B-OMe), 3.84 (3H, s, A-OMe), 3.88 (1H, t, J = 5.5 Hz, Bγ-OH), 4.04 (2H, m, Aβ), 4.21 (2H, dt, J = 5.5, 1.5 Hz, Bγ), 4.55 (1H, d, J = 3.7 Hz, Aα-OH), 4.98 (1H, dt, J = 7.5, 3.7 Hz, Aα), 6.27 (1H, dt, J = 15.9, 5.5 Hz, Bβ), 6.52 (1H, dt, J = 15.9, 1.5 Hz, Bα), 6.80 (1H, d, J = 8.0 Hz, A5), 6.90 (2H, dd, J = 8.1, 1.2 Hz, B6), 6.91 (1H, d, J = 8.1 Hz, B5), 6.92 (1H, dd, J = 8.2, 1.8 Hz, A6), 7.06 (1H, d, J = 1.2 Hz, B2), 7.12 (1H, d, J = 1.8 Hz, A-2); δ_C 56.2 (A-OMe), 56.3 (B-OMe), 63.25 (Bγ), 72.53 (Aα), 75.55 (Aβ), 110.9 (B2), 110.9 (A2), 115.3 (B5), 115.4 (A5), 119.8 (A6), 120.3 (B6), 129.0 (Bb), 130.1 (Bα), 131.9 (B1), 134.0 (A1), 146.8 (A4), 148.1 (A3), 149.1 (B4), 150.8 (B3).

*β-(Sinapyl alcohol) ether of guaiacyl glycol [1-(4-hydroxy-3-methoxyphenyl)-2-[2,6-dimethoxy-4-(3-hydroxypropenyl)phenoxy]-ethanol (**31b**)]:* δ_H 3.69 (1H, m, Aβ1), 3.81 (3H, s, B-OMe), 3.87 (6H, s, A-OMe), 3.91 (1H, d, J = 5.7 Hz), 4.22 (3H, m, Aβ2 and Bγ's), 4.41 (1H, d, J = 2.1 Hz, Aα-OH), 4.81 (1H, dt, J = 9.2, 2.5 Hz, Aα), 6.37 (1H, dt, J = 15.8, 5.2 Hz, Bβ), 6.55 (1H, dt, J = 15.8, 1.3 Hz, Bα), 6.77 (2H, s, B2/6), 6.78 (1H, d, J = 8.2 Hz, A5), 6.83 (1H, dd, J = 8.2, 2.1 Hz, A6), 7.02 (1H, d, J = 2.1 Hz, A-2); δ_C 56.2 (A-OMe), 56.5 (B-OMe's), 63.2 (Bγ), 72.9 (Aα), 80.5 (Aβ), 104.5 (B2/6), 110.7 (A2), 115.4 (A5), 119.8 (A6), 130.0 (Bα), 130.6 (Bβ), 132.9 (A1), 134.3 (B1), 137.3 (B4), 146.8 (A4), 148.1 (A3), 154.0 (B3/5).

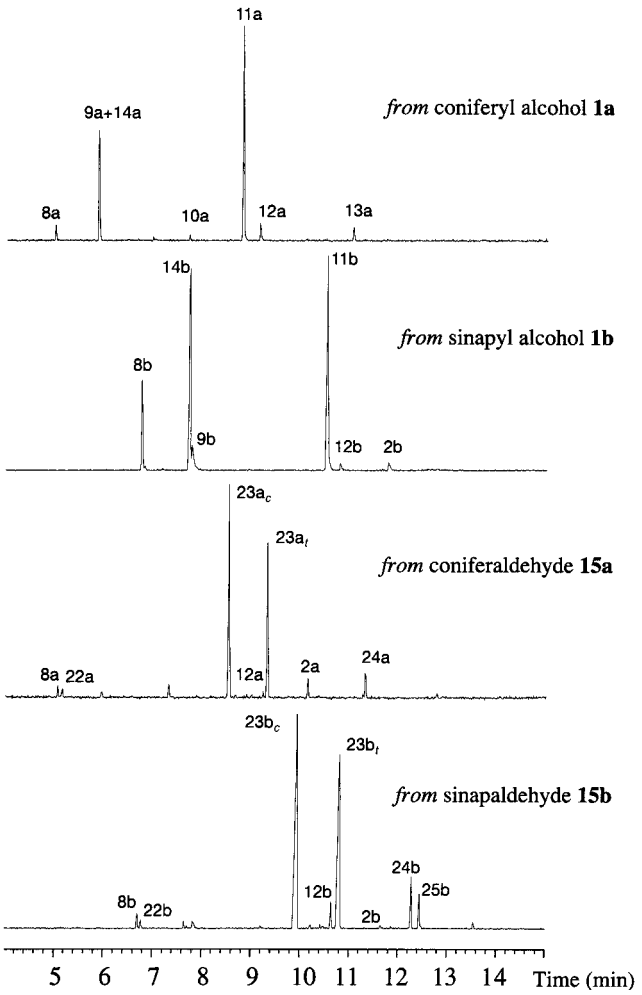

Figure 1. Total-ion chromatograms of DFRC products from coniferyl and sinapyl alcohols **1a** and **1b**, coniferaldehyde **15a**, and sinapaldehyde **15b**.

RESULTS AND DISCUSSION

DFRC Products from Cinnamyl Alcohols and Cinnamaldehydes. Coniferyl alcohol **1a**, sinapyl alcohol **1b**, coniferaldehyde **15a**, and sinapaldehyde **15b** were subjected to the DFRC procedure. Although such units in lignins are completely etherified, reactions on these phenolic models helped to elucidate some DFRC pathways. GC/MS chromatograms of DFRC products from these model compounds are shown in Figure 1, structures in Figures 2 and 3. Peaks were identified by comparison of their GC retention times with those of authentic compounds and/or their mass spectral data (Lu and Ralph, 1998a). The major products shown in Figures 2 and 3 have also been isolated and their structures authenticated by NMR (see Experimental Procedures). The major DFRC products from coniferyl alcohol **1a** were 4-acetoxyguaiacylcyclopropane (**14a**) and the guaiacylpropyl bromide **11a** accompanied by minor components **8a**−**10a** and **12a**−**13a**. Compound **14a** coeluted with a small amount of isoeugenol acetate

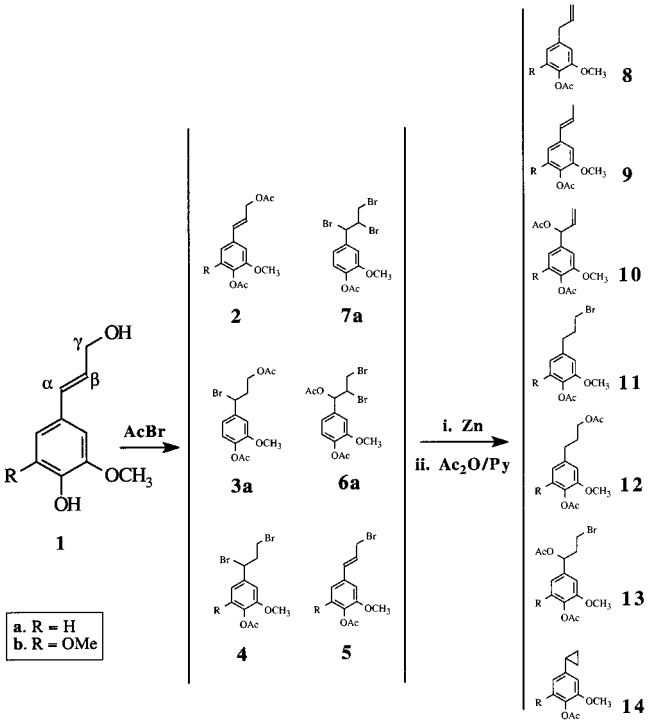

Figure 2. DFRC reactions of coniferyl alcohol **1a** and sinapyl alcohol **1b**.

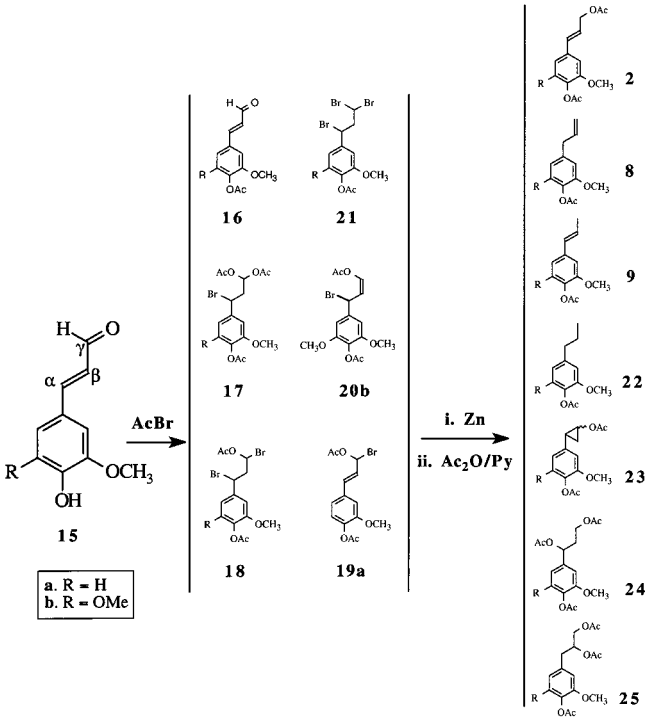

Figure 3. DFRC reactions of coniferaldehyde **15a** and sinapaldehyde **15b**.

(**9a**), which could be detected in the NMR of a fraction from the monomer mixture of the DFRC product from coniferyl alcohol and could be separated using an extended GC temperature program (Lu and Ralph, 1998a). The major monomers from sinapyl alcohol **1b** were the analogous compounds **8b**, **14b**, and **11b**. Compounds **14b** and **9b** were resolved in this case.

The major DFRC monomers from coniferaldehyde **15a** and sinapaldehyde **15b** were *cis*- and *trans*-arylcyclopropyl acetates **23** (Figures 1 and 3). The minor acetylated products from sinapaldehyde (compounds **12b**, **24b**, and **25b**) were produced in higher yield than their counterparts (compounds **12a**, **24a**, and **25a**) from coniferaldehyde. Compound **25a** was barely detected in the GC chromatogram of DFRC products from coniferaldehyde.

Table 1. Products and Their Distributions by Weight from AcBr Treatment of Compounds 1 and 15[a]

substrate	wt distribution of AcBr treatment intermediates (%)
coniferyl alcohol (**1a**)	88 (**4a**), 9 (**6a**), 3 (**3a**), trace (**2a**+ **5a**)
sinapyl alcohol (**1b**)	90 (**4b**), 5 (unknown), 5 (**5b**), trace (**2b**)
coniferaldehyde (**15a**)	84 (**18a**), 11 (**16a**+**17a**+**19a**), 5 (**21a**)
sinapaldehyde (**15b**)	87 (**18b**), 9 (**16b**+**17b**+**20b**), 4 (**21b**)

[a] Identified products represented >90% of the total product.

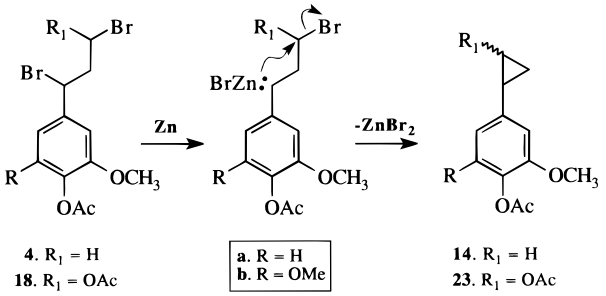

Figure 4. Formation of arylcyclopropane compounds **14** and **23**.

To understand the formation of DFRC monomeric products from cinnamyl alcohols **1** and cinnamaldehydes **15**, the intermediates produced during acetyl bromide (AcBr) treatment (Figures 2 and 3) were isolated by preparative TLC and identified by NMR (see Experimental Procedures). The relative proportions by weight among those intermediates are listed in Table 1. 4-Hydroxycinnamyl alcohols **1** (Figure 2) resulted primarily in aryl-1,3-dibromopropanes **4**, presumably formed through HBr addition to the corresponding free-phenolic precursor of cinnamyl bromide **5**, formed from their alcohols, followed by acetylation. Minor compounds isolated were **3a** and **6a**. Compound **5b** was isolated as a minor component (~5%) in the major **4b** fraction (total = 200 mg). Tiny amounts of AcBr treatment products from sinapyl alcohol remain unknown. No syringyl compounds **3b** or **6b** could be isolated or detected by NMR. Coniferaldehyde **15a** and sinapaldehyde **15b** reacted with AcBr in acetic acid in a similar way to the alcohols, resulting in compounds **18** as major products and **16**, **17**, and **21** as minor products (Figure 3). The only difference was that **19a** was detected in the minor fraction of AcBr treatment products from coniferaldehyde **15a**, but **20b** was observed in the AcBr treatment products from sinapaldehyde **15b**.

Once AcBr treatment intermediates were known, chemical pathways leading to the major DFRC products became easier to understand (Figures 2 and 3). Products **11** were formed by Zn reduction of the benzyl bromides of intermediates **4**. Cyclopropanes **14** were also produced from **4** (Figure 4) following a known reaction pathway (Corbin et al., 1964). Products **8** and **9** may come from Zn reduction of intermediates **5** and/or **7/6**, compound **10a** from **6a**, and compound **12** from **3**. Finally, **13a** probably resulted from partial hydrolysis of the benzyl

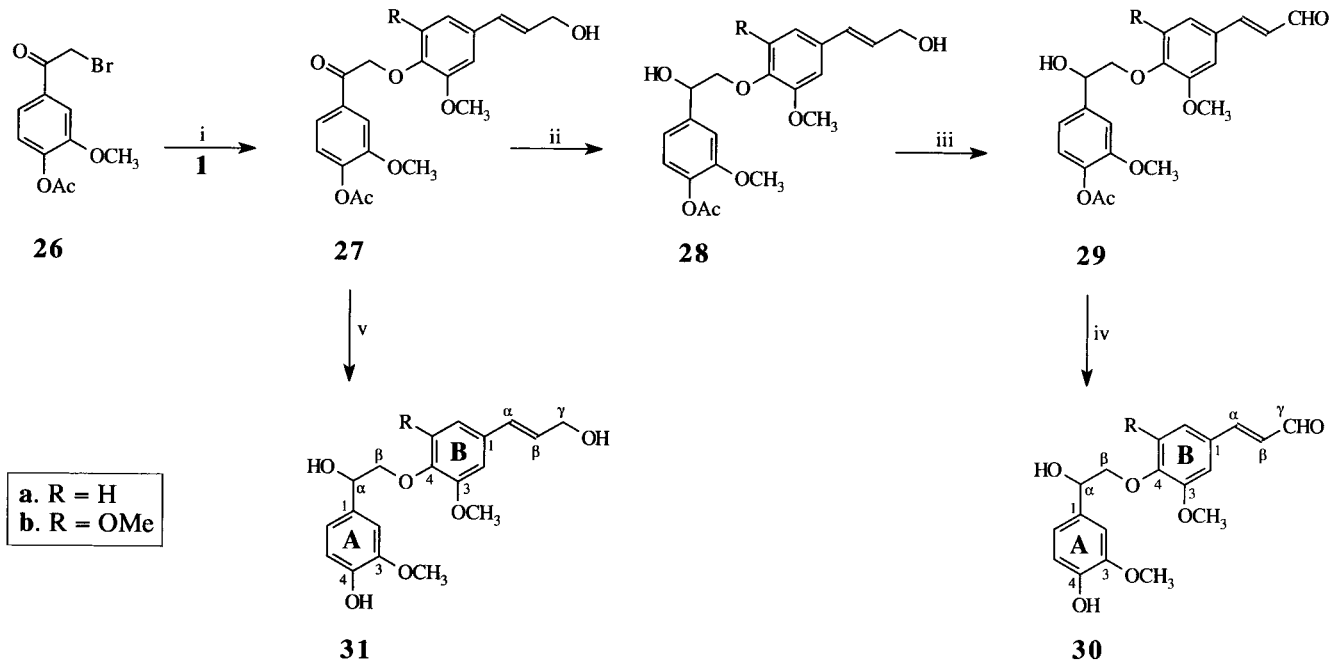

Figure 5. Scheme for syntheses of 4-O-etherified cinnamyl alcohol/cinnamaldehyde end-group model compounds **30** and **31**: (i) K_2CO_3, acetone; (ii) $NaBH_4$, EtOAc; (iii) 1,4-benzoquinone, diglyme, 120 °C; (iv) NaOH, EtOH; (v) $NaBH_4$, EtOH.

bromide on intermediate **4a** followed by acetylation. The AcBr treatment intermediates from the aldehydes **15** suggest that the major products **23** were from intermediates **18** through Zn reduction (Figure 4). Products **8** and **9** may result from **21**, **16**, and/or **19**/**20**. Minor products **24** could be from intermediates **17** or **18,** but how product **25b** has been formed remains unknown. Products **2** were from intermediates **16** by Zn reduction followed by acetylation.

DFRC Monomers from Cinnamyl Alcohol and Cinnamaldehyde End Groups of *β*-Aryl Ether Models 30 and 31. Four *β*-aryl ether lignin model compounds containing etherified cinnamyl end groups, which are better representatives of their structures in lignins, were synthesized according to standard methods as shown in Figure 5. Hydroxycinnamyl alcohols **1** were synthesized by reduction of hydroxycinnamaldehydes **15** with sodium triacetoxyborohydride (Lu and Ralph, 1998d) and condensed with bromo-4-acetoxy-3-methoxyacetophenone (**26**). The resultant ketone **27** was reduced with sodium borohydride in ethyl acetate to the corresponding benzyl alcohol **28**. Benzoquinone oxidation (Kulkarni and Sebastian, 1990) oxidized the primary alcohol group in **28** to give the aldehyde **29**. Other steps shown in Figure 5 have already been described (Ralph and Young, 1981; Helm and Ralph, 1992). A simple two-carbon side chain on the A moiety was used instead of the typical three-carbon one of lignin models because DFRC products from cinnamyl alcohol/cinnamaldehyde end groups can be readily found and the A side chain was not expected to significantly affect the reaction course. In this case, the only DFRC product from the A moieties of models **30** and **31** was the 4-acetoxy-3-methoxystyrene; the rest of the products came from the corresponding end groups (B moiety). Figure 6 shows total ion chromatograms of DFRC products from models **30** and **31**. By comparing Figure 6 with Figure 1, it was found that products from 4-O-etherified cinnamyl end groups were similar to those from the corresponding cinnamyl alcohols/cinnamaldehydes with free-phenolic groups. However, the proportions of the products were different, especially for cinnamyl alcohol end groups. Model **31a** produced more

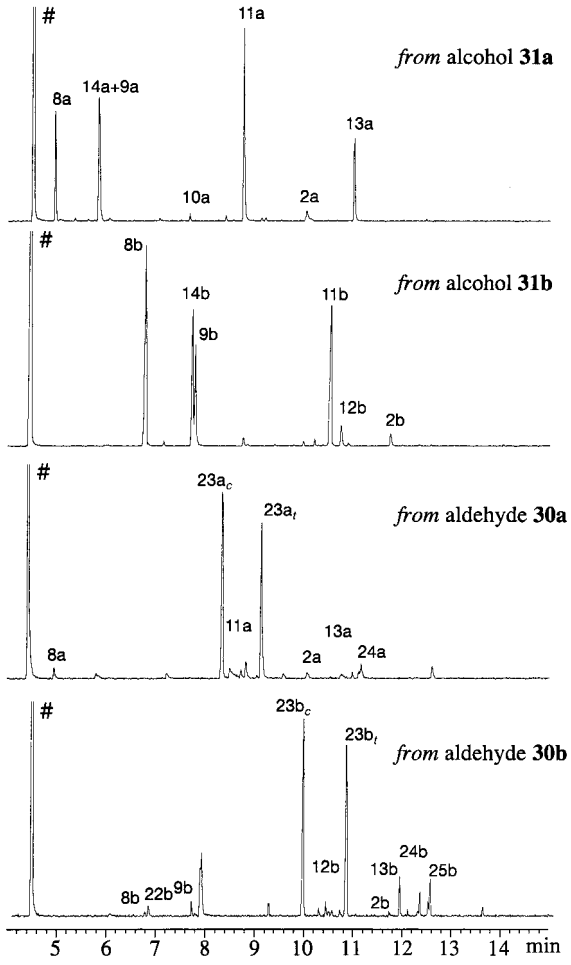

Figure 6. Total-ion chromatograms of DFRC products from models **30** (containing 4-O-etherified cinnamaldehydes) and **31** (containing 4-O-etherified cinnamyl alcohols): (#) 4-acetoxy-3-methoxystyrene from the A moiety.

8a, **13a**, and **9a** compared to coniferyl alcohol **1a**. Similarly, model **31b** gave rise to more **8b** and **9b** compared to sinapyl alcohol **1b**. Model **30** had patterns of products similar to those from the corresponding aldehydes **15**, although small variations were found in the case of **30b**, from which a significant amount of one unknown compound **X** and **13b** were produced by the DFRC method.

Formation and Identification of the Arylcyclopropane Compounds 14 and 23. The major DFRC monomers from cinnamaldehyde end groups were diagnostic arylcyclopropyl acetates **23** (Figure 3), whereas significant amounts of arylcyclopropanes **14** (Figure 2) were produced from cinnamyl alcohol end groups (Figure 1). Each diastereoisomer of compounds **23** was isolated by reverse-phase TLC following normal-phase TLC from preparative-scale DFRC of cinnamaldehydes **15**. Identification of **23** isomers was made by the usual series of NMR experiments (^{1}H, ^{13}C, DEPT, and 2D gradient-enhanced HMQC and HMBC). Two low-field multiplet signals around δ_H 1.2−1.3 in the ^{1}H NMR spectra, and the corresponding δ_C 11.4−11.5 methylene signals in the ^{13}C NMR spectra, indicated the presence of cyclopropane protons in compounds **23**. Singlets integrating for three protons at δ_H 1.8−2.0 indicated a side-chain (aliphatic) acetate attached to tertiary carbons. In HMBC experiments, C-1 on the aromatic ring correlated with all of the protons on the side chain, an occurrence not encountered in typical aryl-*n*-propyl side chains. All of the NMR and mass spectral data of compounds **23** were consistent with arylcyclopropyl acetates. Isomers were assigned on the basis of the higher ^{13}C shift of C_α and C_γ (Breitmaier and Voelter, 1987) accompanied by a lower proton shift of H_γ due to shielding by the aromatic ring in the *trans*-isomer.

Compounds **14** were isolated (in the same way as was used for isolating compounds **23**) from preparative DFRC of cinnamyl alcohols **1**. In the proton NMR spectra of **14**, two multiplet peaks at δ_H 0.7 and 0.9, integrating for two protons each, indicated the presence of two pairs of cyclopropane protons. Those protons correlated with only one carbon peak in HMQC experiments, suggesting a symmetry. Again, C-1 of the aromatic ring correlated with all side-chain protons in HMBC experiments. Thus, compounds **14** were identified as arylcyclopropanes.

Phenylcyclopropane has been obtained by treatment of 1-phenyl-1,3-bromopropane with a Zn−Cu couple in dimethylformamide (Corbin et al., 1964). Similar ring closure was also observed when comparable reducing conditions were applied to 1,3-dihilades (Rifi, 1967). Hence, compounds **14** and **23** likely resulted from the 1,3-dibromides **4** and **18** formed in the AcBr treatment step. To confirm this pathway, **4** and **18** were isolated from AcBr treatment of hydroxycinnamyl alcohols **1** and hydroxycinnamaldehydes **15**. When **4a** was treated with Zn dust in dioxane/acetic acid/water mixed solvent, as in the reductive step of the DFRC procedure, compounds **11a** and **14a** were produced, accompanied by small amounts of hydrolysis product, 3-bromo-1-(4-acetoxy-3-methoxy)phenylpropanol, from which **13a** was formed by acetylation. Compounds **23** were produced analogously when compounds **18** were treated with Zn under DFRC conditions (Figure 4).

CONCLUSIONS

Diagnostic products were formed from cinnamyl alcohol and cinnamaldehyde end groups in lignins following DFRC treatment. The reactions are not as clean as the ether-cleaving reactions that form the basis of the DFRC method but, nevertheless, provide valuable markers for studying end groups in lignins. Cinnamaldehyde end groups produce characteristic arylcyclopropyl acetates, so the DFRC method could find value in the understanding of compositional changes in mutant and transgenic plants where aldehyde buildup is suspected. Cinnamyl alcohol groups produce a mixture of arylpropenes and 1-aryl-3-bromopropanes, along with more diagnostic arylcyclopropanes. Coniferyl alcohol end groups also form 1-acetoxy-1-aryl-3-bromopropanes. Although the product mixtures are more complex, the production of relatively diagnostic "fingerprint" products from cinnamyl alcohol end groups also allows the DFRC method to provide useful data on these features of lignins.

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