Lignin–Hydroxycinnamyl Model Compounds Related to Forage Cell Wall Structure. 2. Ester-Linked Structures

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Model compounds which depict *p*-coumarate and ferulate attachment at the α- and γ-positions and at the phenolic position of the β-*O*-4 interunit linkage of lignin have been synthesized and characterized by NMR spectroscopy. The α-ester trimers were prepared by nucleophilic attack of ferulic and *p*-coumaric acids on the quinone methide generated from guaiacylglycerol β-guaiacyl ether under slightly basic conditions. The γ-esters were prepared via 4-acetoxycinnamoylation of 1-(4-acetoxy-3-methoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)propanone. Reduction with zinc borohydride was selective for the erythro diacetates, and the threo isomers were obtained by nonselective reduction with sodium cyanoborohydride. Deacetylation with piperidine provided the unprotected γ-trimers. The phenolate esters were synthesized from the acetates of purified *threo*- and *erythro*-1-[4-(benzyloxy)-3-methoxyphenyl]-1,3-dihydroxy-2-(2-methoxyphenoxy)propane. Debenzylation and 4-acetoxycinnamoylation afforded the peracetylated hydroxycinnamate phenolic esters of guaiacylglycerol β-guaiacyl ether.

INTRODUCTION

The cross-linking of lignin and hemicellulose through ferulic and *p*-coumaric acids is thought to have a pronounced effect on forage cell wall development and degradation (Bacic et al., 1988; Fry and Miller, 1989; Lewis and Yamamoto, 1990). As part of our efforts to characterize this proposed interaction, we have prepared numerous synthetic analogs to the lignin–hydroxycinnamic acid–polysaccharide cross-link (Helm et al., 1992; Helm and Ralph, 1992a; Ralph et al., 1992b). Our attention has focused mainly on the predominant β-*O*-4 interunit of lignin as the most probable site of hydroxycinnamic acid attachment to the lignin macromolecule and the 5-position of L-arabinofuranosyl units as the site of esterification to hemicelluloses. The possibility that hydroxycinnamic acids can be esterified to lignin and therefore not acylated to polysaccharides also exists (Scalbert et al., 1985; Kondo et al., 1990; Lam et al., 1990). Indeed, there is strong evidence for the esterification of hydroxycinnamic acids to the γ-position of native lignins (Shimada et al., 1971; Nakamura and Higuchi, 1978), and α-esterification has been demonstrated to occur in model systems (Scalbert et al., 1986). As a companion to our work with etherified lignin–hydroxycinnamic acid etherified structures (Helm and Ralph, 1992b), we have developed synthetic techniques for the preparation and characterization of esterified structures related to the predominant β-*O*-4 interunit linkage of lignin.

EXPERIMENTAL METHODS

Standard reaction procedures and processing protocols are as described previously (Ralph et al., 1992a). NMR spectra were recorded on a Bruker AMX-360 instrument at 300 K utilizing the experiments described earlier (Helm and Ralph, 1992b).

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α-Esters. *A. Peracetates.* A threo/erythro mixture of **1** (405.5 mg, 1.27 mmol) was converted to the quinone methide (**2**) with TMSBr as described (Helm and Ralph, 1992b). 4-Acetoxyferulic acid (Hatfield et al., 1991; 452.2 mg, 1.91 mmol) was dissolved in DMSO (3 mL), and to this solution was added a catalytic amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 3 μL, 0.02 mmol). The carboxylic acid solution was added dropwise (via a pasteur pipet) to the stirred solution of **2**. The yellow color indicative of the quinone methide disappeared over the course of 3 h, and the solution was left overnight without stirring. TLC ($CHCl_3$–EtOAc 1:1) after 15 h indicated conversion to two products (R_f ca. 0.45). The solution was cooled in an ice–water bath, and Ac_2O (360 μL) and 4-(dimethylamino)pyridine (DMAP; 352 mg) were added in rapid succession to the stirred reaction mixture. TLC ($CHCl_3$–EtOAc 1:1) indicated conversion to the crude peracetates was complete in less than 15 min. The solution was transferred to a separatory funnel and washed in succession with cold aqueous 3% HCl (1×) and aqueous NH_4Cl (150 g/L; 2×). Processing afforded a crude solid (723 mg) which was purified by silica gel chromatography: 40 g of silica, packed with $CHCl_3$, and eluted with $CHCl_3$ (250 mL) followed by $CHCl_3$–EtOAc (50:1, 300 mL). The erythro isomer of 3-acetoxy-1-(4-acetoxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propyl 4-acetoxyferulate (**6**; 432.7 mg) eluted first followed by an intermediate threo/erythro mixture (61.3 mg) and **6**-*threo* (90% threo, 125.0 mg). The overall yield of **6** was 78% with a threo:erythro ratio of about 25:75.

The preparation of the peracetylated α-(*p*-coumarate) esters (**5**) was accomplished in the same way with 4-acetoxy-*p*-coumaric acid (Helm et al., 1992) used in the addition step. The silica-purified isomers of **5** were isolated in 79% yield with a threo:erythro ratio of ca. 25:75.

B. Free. The α-esterification procedure used was as described under Peracetates with ferulic and *p*-coumaric acids used in the addition step. The crude product, instead of being submitted to acetylation, was diluted with CH_2Cl_2 and washed with aqueous NH_4Cl (3×) and processed to afford an off-white foam. Purification by silica gel chromatography (40 g of silica, $CHCl_3$–EtOAc 2:1) gave the erythro α-esters followed by the threo α-esters and subsequently 1-*threo*. Yields were typically 40–50% with the threo:erythro ratio being about 20:80.

γ-Esters. *A. Precursors. 1. 1-(4-Acetoxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)ethanone* (**8**). 4-Acetoxy-α-bromo-3-methoxyacetophenone (Helm and Ralph, 1992b; **7**; 4.08 g, 14.2 mmol) was dissolved in acetone (50 mL), and guaiacol (1.80 g, 14.5 mmol), K_2CO_3 (powdered, 2.07 g, 15.0 mmol), and KI (50

mg) were added. The solution was stirred at reflux for 8 h, at which time TLC ($CHCl_3$–EtOAc 19:1) indicated almost complete conversion to a slower moving material. The solution was filtered and the filtrate evaporated to a syrup. The syrup was dissolved in CH_2Cl_2 and washed successively with aqueous $NaHCO_3$ (2×) and aqueous NaCl (1×). Standard processing gave a syrup which crystallized upon standing. Washing the crystals with 95% EtOH gave **8** (3.89 g, 83%), which could be recrystallized from absolute EtOH: mp 102–105 °C; ^{1}H NMR (acetone-d_6) δ 5.429 (1 H, s, β); ^{13}C NMR (acetone-d_6) δ 20.44 (Ac), 56.21 and 56.44 (OMe), 72.50 (β), 112.66 (A-2), 113.63 (B-2), 115.72 (B-5), 121.54 (B-6), 122.13 (A-6), 122.79 (B-1), 123.99 (A-5), 134.59 (A-1), 145.22 (A-4), 148.97 (B-4), 150.88 (B-3), 152.58 (A-3), 168.63 (Ac), 194.31 (α).

2. 1-(4-Acetoxy-3-methoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)propanone (**9**). Crystalline **8** (1.04 g, 3.16 mmol) was dissolved in dioxane (30 mL), and powdered K_2CO_3 (3.27 g) was added followed by aqueous formaldehyde (37 wt %, 455 μL, 3.0 mmol). The solution was stirred vigorously for 4.5 h, at which time TLC ($CHCl_3$–EtOAc 9:1) indicated almost complete conversion to a slower moving material. Prolonged exposure to the hydroxymethylation conditions leads to β,γ-dehydration. The reaction mixture was filtered, and the filtrate was evaporated to a syrup. The syrup was washed with aqueous NH_4Cl (2×) and processed in the usual manner. Purification by silica gel chromatography ($CHCl_3$–EtOAc 9:1) afforded **9** (919 mg, 81%), which crystallized from the syrup as small spherulites: mp 81–83 °C; ^{1}H NMR (acetone-d_6) δ 4.088 (2 H, m, γ_1 and γ_2), 5.545 (1 H, t, J = 5 Hz, β); ^{13}C NMR (acetone-d_6) δ 20.44 (Ac), 56.12 and 56.35 (OMe), 63.90 (γ), 84.22 (β), 113.43 (A2), 113.73 (B-2), 117.45 (B-5), 121.60 (B-6), 122.81 (A-6), 123.34 (B-1), 123.80 (A-5), 135.27 (A-1), 145.05 (A-4), 148.29 (B-4), 151.13 (B-3), 152.36 (A-3), 168.60 (Ac), 196.80 (α).

B. Acylation. Compound **9** (0.81 mmol) was dissolved in CH_2Cl_2 (5 mL), and freshly prepared crystalline 4-acetoxycinnamoyl chloride (Helm et al., 1992; 0.91 mmol) was added. The solution was cooled in an ice–water bath, and DMAP (0.95 mmol) was added. The solution was removed from the ice–water bath and stirred under TLC ($CHCl_3$–EtOAc 19:1) indicated complete conversion to a faster moving material (1 h). The solution was diluted with CH_2Cl_2 and washed successively with cold aqueous 3% HCl and aqueous NH_4Cl. Processing and silica gel chromatography ($CHCl_3$–EtOAc 19:1) gave 2-(4-acetoxy-3-methoxybenzoyl)-2-(2-methoxyphenoxy)ethyl 4-acetoxy-*p*-coumarate (**10**) and 2-(4-acetoxy-3-methoxybenzoyl)-2-(2-methoxyphenoxy)ethyl 4-acetoxyferulate (**11**) as white powders in yields of 88–97%: ^{1}H NMR (acetone-d_6) δ (**10**) 4.620 (1 H, dd, $J_{\gamma1,\beta}$ = 6.5 Hz, $J_{\gamma1,\gamma2}$ = 12.0 Hz, γ_1), 4.806 (1 H, dd, $J_{\gamma2,\beta}$ = 3.8 Hz, γ_2), 5.913 (1 H, dd, β), 6.482 (1 H, d, $J_{C8,C7}$ = 16.0 Hz, C-8), 7.600 (1 H, d, C-7); (**11**) 4.621 (1 H, dd, $J_{\gamma1,\beta}$ = 6.5 Hz, $J_{\gamma1,\gamma2}$ = 12.0 Hz, γ_1), 4.808 (1 H, dd, $J_{\gamma2,\beta}$ = 3.8 Hz, γ_2), 5.914 (1 H, dd, β), 6.523 (1 H, d, $J_{F8,F7}$ = 16.0 Hz, F-8), 7.577 (1 H, d, F-7); ^{13}C NMR (acetone-d_6) δ (**10**) 64.97 (γ), 80.56 (β), 118.32 (C-8), 145.09 (C-7), 166.76 (C-9), 195.13 (α); (**11**) 64.93 (γ), 80.54 (β), 118.44 (F-8), 145.35 (F-7), 166.81 (F-9), 195.11 (α).

C. Reduction. 1. $Zn(BH_4)_2$. Compound **10** or **11** (0.7 mmol) was dissolved in EtOAc (5 mL) and cooled to 0 °C. Ethereal $Zn(BH_4)_2$ (Helm and Ralph, 1992b; ca. 0.15 M, 5 mL) was added, and the reaction was monitored by TLC, which indicated the reaction was done within 1 h. The mixture was quenched by the addition of H_2O (2 mL) followed by HOAc (200 μL), diluted with EtOAc, and washed with aqueous NH_4Cl (2×). Processing and silica gel chromatography ($CHCl_3$–EtOAc 9:1) gave the α-hydroxy diacetates **12** and **13** in 90–92% yield with erythro selectivity being greater that 90%: ^{1}H NMR (acetone-d_6) δ (**12**-*erythro*) 4.451 (1 H, dd, $J_{\gamma1,\beta}$ = 3.7 Hz, $J_{\gamma1,\gamma2}$ = 11.8 Hz, γ_1), 4.523 (1 H, dd, $J_{\gamma2,\beta}$ = 6.3 Hz, γ_2), 4.704 (1 H, m, β), 4.796 (d, $J_{OH,\alpha}$ = 4.4 Hz, α-OH), 5.088 (bt, 1 H, α), 6.405 (1 H, d, $J_{C8,C7}$ = 16.1 Hz, C-8), 7.497 (1 H, d, C-7); (**13**-*erythro*) 4.452 (1 H, dd, $J_{\gamma1,\beta}$ = 3.7 Hz, $J_{\gamma1,\gamma2}$ = 11.8 Hz, γ_1), 4.519 (1 H, dd, $J_{\gamma2,\beta}$ = 6.2 Hz, γ_2), 4.702 (1 H, m, β), 4.788 (d, $J_{OH,\alpha}$ = 4.4 Hz, α-OH), 5.088 (bt, 1 H, α), 6.440 (1 H, d, $J_{C8,C7}$ = 16.0 Hz, F-8), 7.468 (1 H, d, F-7); ^{13}C NMR (acetone-d_6) δ (**12**-*erythro*) 63.95 (γ), 73.16 (α), 83.22 (β), 118.83 (C-8), 144.36 (C-7), 166.79 (C-9); (**13**-*erythro*) 63.93 (γ), 73.14 (α), 83.18 (β), 118.91 (F-8), 144.79 (F-7), 166.83 (F-9).

2. $NaBH_3CN$. The reductions were performed essentially as described by Borch et al. (1971). The γ-(4-acetoxycinnamates) **10** and **11** (0.24 mmol) were dissolved in THF–H_2O (6:1, 3.5 mL), and a small amount of bromocresol green (pH indicator) was added. The addition of $NaBH_3CN$ (180 mg) caused the solution to turn deep blue, and aqueous HCl (3%) was added dropwise with stirring to return the solution to a yellow color. The aqueous acid was then added when necessary to maintain the yellow color. When TLC ($CHCl_3$–EtOAc 19:1) indicated the reaction was complete (10 h), the solution was diluted with CH_2Cl_2 and washed with aqueous NH_4Cl (3×). Purification by silica gel chromatography (26 g of silica, $CHCl_3$–EtOAc 9:1) gave threo/erythro mixtures of **12** and **13** in 83–91% yield: ^{1}H NMR (acetone-d_6) δ (**12**-*threo*) 4.173 (1 H, dd, $J_{\gamma1,\beta}$ = 6.3 Hz, $J_{\gamma1,\gamma2}$ = 11.9 Hz, γ_1), 4.435 (1 H, dd, $J_{\gamma2,\beta}$ = 3.7 Hz, γ_2), 4.640 (1 H, dt, β), 4.752 (d, $J_{OH,\alpha}$ = 4.2 Hz, α-OH), 5.084 (1 H, bm, α), 6.456 (1 H, d, $J_{C8,C7}$ = 16.1 Hz, C-8), 7.536 (1 H, bd, C-7); (**13**-*threo*) 4.169 (1 H, dd, $J_{\gamma1,\beta}$ = 6.2 Hz, $J_{\gamma1,\gamma2}$ = 11.9 Hz, γ_1), 4.435 (1 H, dd, $J_{\gamma2,\beta}$ = 3.7 Hz, γ_2), 4.644 (1 H, dt, β), 4.752 (d, $J_{OH,\alpha}$ = 4.2 Hz, α-OH), 5.08 (1 H, bm, α), 6.488 (1 H, d, $J_{F8,F7}$ = 16.0 Hz, F-8), 7.507 (1 H, bd, F-7); ^{13}C NMR (acetone-d_6) δ (**12**-*threo*) 64.28 (γ), 73.58 (α), 83.80 (β), 118.73 (F-8), 144.51 (F-7), 166.68 (F-9); (**13**-*threo*) 64.27 (γ), 73.56 (α), 83.73 (β), 118.81 (F-8), 144.94 (F-7), 166.72 (F-9).

D. Acetylation/Deacetylation. Acetylations were performed in CH_2Cl_2 with Ac_2O–DMAP as described (Ralph et al., 1992b) and were essentially quantitative. Deacetylation was accomplished with 1 M piperidine in THF–H_2O (10:1, 30 mg/mL; Helm et al., 1992) with a 3-h reaction time being sufficient. The deacetylated products were purified by silica gel chromatography ($CHCl_3$–EtOAc 2:1) to afford **14** and **15** in 60–65% yield.

Phenolic Esters. *A. Threo Precursors.* 1-[4-(Benzyloxy)-3-methoxyphenyl]-2-(2-methoxyphenoxy)propane-1,3-diol (**18**; Landucci et al., 1981; Ralph and Young, 1981), a 70:30 threo:erythro mixture [4.41 g, 10.8 mmol; obtained from the $NaBH_4$ reduction of the corresponding α-ketone in aqueous ethanol; see, for example, Barrele et al. (1989) and Ralph and Helm (1991)] was acetylated overnight in pyridine (10 mL)–acetic anhydride (7 mL). The reaction was quenched with absolute EtOH (5 mL) and diluted with CH_2Cl_2. The mixture was washed successively with cold aqueous HCl (3% by vol, 2×) and aqueous NH_4Cl. The solution was further processed to afford an amber syrup which was submitted directly to debenzylation.

The crude diacetate was dissolved in 95% EtOH (200 mL), and Pd/C (5% by wt, 505 mg) was added. A balloon filled with H_2 gas was placed on top of the flask, and the solution was stirred vigorously; additional gas was added when necessary. TLC ($CHCl_3$–EtOAc 4:1) indicated that the reaction was complete after 15 h. The solution was then filtered through a 0.2-μm nylon membrane filter, and the filtrate was evaporated to afford a threo/erythro mixture of 1,3-diacetoxy-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane as an amber syrup (3.97 g, 91%). A portion of the syrup (302.6 mg) was further purified by silica gel chromatography (40 g of silica, $CHCl_3$–EtOAc 19:1) to provide the **19**-*threo* (186.2 mg, 62% yield, 92% threo): ^{1}H NMR (acetone-d_6) δ 3.957 (1 H, dd, $J_{\gamma1,\beta}$ = 5.6 Hz, $J_{\gamma1,\gamma2}$ = 11.9 Hz, γ_1), 4.211 (1 H, dd, $J_{\gamma2,\beta}$ = 3.6 Hz, γ_2), 4.766 (1 H, m, β), 6.039 (1 H, d, $J_{\alpha,\beta}$ = 7.1 Hz, α); ^{13}C NMR (acetone-d_6) δ 20.58 and 20.97 (Ac), 56.21 and 56.30 (OMe), 63.86 (γ), 75.87 (α), 80.99 (β), 111.89 (A-2), 113.77 (B-2), 115.64 (A-5), 119.13 (B-5), 121.19 (A-6), 121.67 (B-6), 123.57 (B-1), 129.17 (A-1), 147.71 (A-4), 148.26 (A-3), 149.31 (B-4), 169.95 and 170.66 (Ac).

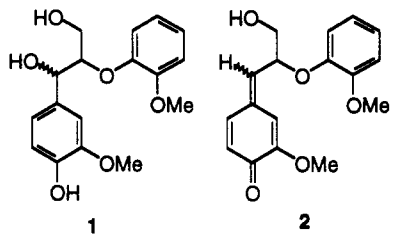
B. Erythro Precursors. *erythro*-Ethyl 3-[4-(benzyloxy)-3-methoxyphenyl]-3-hydroxy-2-(2-methoxyphenoxy)propionate (**20**-*erythro*; 1.06 g, 2.35 mmol; Nakatsubo et al., 1975) was dissolved in THF (5 mL) and added to a freshly prepared solution of $LiAlH_4$ (300 mg) in THF (35 mL). The solution was refluxed for 80 min and cooled, and the excess hydride was carefully decomposed with H_2O. The mixture was transferred to a separatory funnel, diluted with Et_2O, and washed with aqueous sodium potassium tartrate. The aqueous layer was washed one time with fresh ether, and the ether layers were combined and washed successively with aqueous sodium potassium tartrate and H_2O. Standard processing gave the crude *erythro*-benzyl ether (943.8 mg, 98%). Subsequent acetylation and debenzylation as outlined for the threo isomer gave *erythro*-1,3-diacetoxy-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane (**21**-*erythro*) in 91% overall yield from **20**-*erythro*: ^{1}H NMR (acetone-d_6) δ 3.976 (1 H, dd, $J_{\gamma1,\beta}$ = 4.0 Hz, $J_{\gamma1,\gamma2}$ = 11.8 Hz, γ_1), 4.340 (1 H, dd, $J_{\gamma2,\beta}$

= 6.2 Hz, γ_2), 4.808 (1 H, m, β), 5.992 (1 H, d, $J_{\alpha,\beta}$ = 5.0 Hz, α); ^{13}C NMR (acetone-d_6) δ 20.60 and 20.90 (Ac), 56.20 and 56.29 (OMe), 63.30 (γ), 74.89 (α), 80.40 (β), 112.01 (A-2), 113.78 (B-2), 115.41 (A-5), 119.56 (B-5), 121.28 (A-6), 121.61 (B-6), 123.83 (B-1), 129.07 (A-1), 147.53 (A-4), 148.15 (A-3), 148.49 (B-4), 152.00 (B-3), 169.90 and 170.75 (Ac).

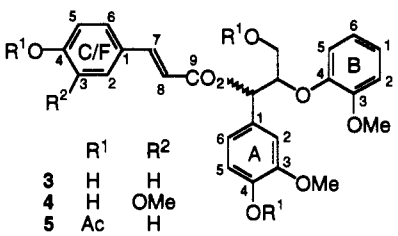
C. Acylation. Diacetate **19** or **21** (235 mg, 0.58 mmol) was dissolved in CH_2Cl_2 (5 mL), and the desired 4-acetoxycinnamoyl chloride (0.66 mmol) was added. The solution was cooled to 0 °C, and DMAP (0.69 mmol) was added with stirring. TLC indicated that the reaction was done within 5 min, but mixtures were typically left for up to 1 h. The solution was subsequently diluted with CH_2Cl_2 and washed successively with aqueous NH_4Cl, cold 3% HCl, and aqueous NaCl. Processing and silica gel chromatography afforded the peracetylated phenolic esters **22** and **23** in 82–92% yield. The yield of the threo isomer was lower (82–84%) as further separation of the threo isomer from the earlier eluting erythro isomer resulted in a lower overall yield but a higher diastereomeric purity (>95% threo).

RESULTS AND DISCUSSION

Synthetic Aspects. The peracetylated α-esters were prepared by the addition of 4-acetoxycinnamic acids to the quinone methide **2** under slightly basic conditions,



1 **2**

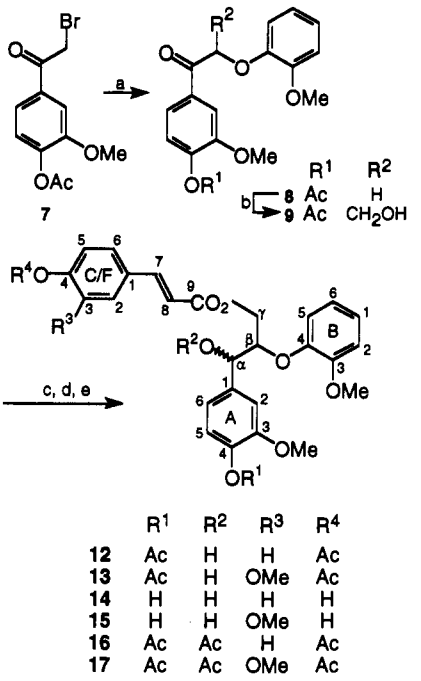


	R^1	R^2
3	H	H
4	H	OMe
5	Ac	H
6	Ac	OMe

which were generated by the addition of a catalytic amount of DBU. This is essentially the same procedure outlined for the α-ether models (Helm and Ralph, 1992b). The subsequent addition of DMAP and acetic anhydride provided a one-pot α-esterification/acetylation sequence which gave the desired peracetates (**5** and **6**) in good yields (78%). Purification by silica gel chromatography gave the racemic threo and erythro isomers, with the erythro isomer predominating (threo:erythro 25:75).

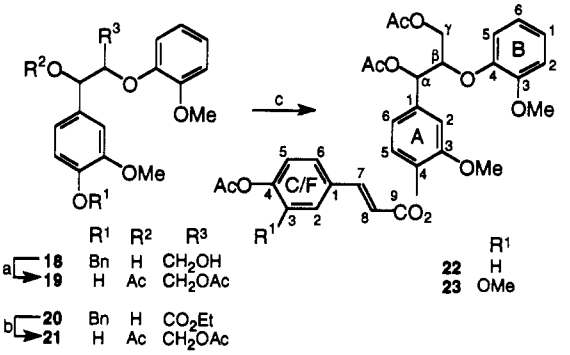
The same procedures were used for preparation of the free α-esters **3** and **4**, except that ferulic and *p*-coumaric acids were used in the addition step and the in situ acetylation was not performed. It is well established that acids add preferentially to quinone methides when in competition with a phenolic hydroxyl (Leary et al., 1977; Scalbert et al., 1986; Sipilä and Brunow, 1991). It is important to note that the yields of free α-esters were significantly less than that for the cinnamoylation/acetylation procedure (45% vs 78%) due to product degradation during reaction processing. Small amounts of 1-*threo* were isolated but no α-ethers were detected. Yields were also in the 40% range when 4-acetoxycinnamic acids were used in the addition step. Thus, the α-esters are not stable in aqueous environments (this instability was noted in the preparation of deuterium-exchanged samples for NMR analysis where degradation was observed

Scheme I. Synthesis of the γ-Esters of Guaiacylglycerol β-Guaiacyl Ether: a, Guaiacol–K_2CO_3; b, H_2CO–K_2CO_3; c, 4-Acetoxycinnamoyl Chloride–DMAP; d, $Zn(BH_4)_2$ or $NaBH_3CN$; e, Ac_2O–DMAP or Piperidine–THF–H_2O



	R^1	R^2	R^3	R^4
12	Ac	H	H	Ac
13	Ac	H	OMe	Ac
14	H	H	H	H
15	H	H	OMe	H
16	Ac	Ac	H	Ac
17	Ac	Ac	OMe	Ac

Scheme II. Synthesis of the β-*O*-4-Based Phenolic Esters: a, Ac_2O–pyr; H_2–Pd/C; b, $LiAlH_4$; Ac_2O–pyr; H_2–Pd/C; c, 4-Acetoxycinnamoyl Chloride



in the spectra). Scalbert et al. (1986) have reported the preparation of **4** using tetrabutylammonium hydroxide as the base. Although no yields or reference to compound instability were discussed, the threo:erythro ratio was in "close proportions". In our hands this procedure provided yields of 35–40% with a threo:erythro ratio of 20:80 (i.e., similar to that of the DMSO/DBU protocol).

The γ-esters were prepared by a variation of the standard route to 1-*threo* (Scheme I; Landucci et al., 1981; Ralph and Young, 1981; Helm and Ralph, 1992b). The bromide **7** was reacted with guaiacol and subsequently submitted to a hydroxymethylation to afford **9**. Crystalline **9** contains only one hydroxyl function at the requisite γ-position. Thus, coupling with 4-acetoxycinnamoyl chlorides provides the appropriate γ-cinnamate functionality. Reduction with $Zn(BH_4)_2$ gave the reduced diacetates **12** and **13**, with the erythro isomer predominating (>90%). The erythro isomers of **14**–**17** were then obtained by either acetylation (Ac_2O–DMAP) or deacetylation (piperidine). The threo isomers were obtained by reduction of **12** and **13** with $NaBH_3CN$ in acidified THF–H_2O. This reduction was nonselective and afforded threo/erythro mixtures in ca.

Table I. Selected ^{1}H NMR Data for the Model Trimer Esters and Their Corresponding Peracetates[a]

compd	C/F-7 ($J_{7,8}$)	C/F-8	α ($J_{\alpha,\beta}$)	β	γ_2 ($J_{\gamma2,\beta}$)	γ_1 ($J_{\gamma1,\beta}$)
3-*threo*	7.503 (16.0)	6.294	6.155 (7.3)	4.581	3.672 (3.7)	3.500 (5.4)
3-*erythro*	7.564 (16.0)	6.345	6.123 (4.8)	4.663	3.788 (5.7)	3.675 (4.8)
4-*threo*	7.487 (15.9)	6.337	6.156 (7.3)	4.582	3.674 (3.8)	3.504 (5.3)
4-*erythro*	7.550 (15.9)	6.388	6.125 (4.8)	4.671	3.785 (5.8)	3.670 (4.8)
5-*threo*	7.625 (16.0)	6.523	6.241 (6.6)	4.893	4.305 (4.1)	4.081 (5.6)
5-*erythro*	7.707 (16.0)	6.607	6.216 (4.7)	4.934	4.408 (6.0)	4.287 (4.4)
6-*threo*	7.599 (16.0)	6.588	6.236 (6.5)	4.894	4.301 (4.1)	4.085 (5.6)
6-*erythro*	7.682 (16.0)	6.645	6.216 (4.7)	4.939	4.402 (6.1)	4.277 (4.4)
14-*threo*	7.474 (16.0)	6.294	4.967 (6.2)	4.558	4.362 (3.4)	4.099 (6.2)
14-*erythro*	7.564 (16.0)	6.345	4.921 (4.9)	4.661	4.458 (6.6)	4.389 (3.6)
15-*threo*	7.454 (15.9)	6.334	4.962 (6.3)	4.557	4.361 (3.5)	4.096 (6.2)
15-*erythro*	7.403 (15.9)	6.278	4.972 (4.9)	4.662	4.459 (6.5)	4.395 (3.7)
16-*threo*	7.565 (16.0)	6.511	6.182 (6.5)	4.876	4.398 (4.0)	4.168 (5.6)
16-*erythro*	7.560 (16.0)	6.447	6.131 (5.1)	4.920	4.484 (5.9)	4.402 (4.2)
17-*threo*	7.540 (16.0)	6.553	6.175 (6.6)	4.878	4.401 (3.9)	4.157 (5.6)
17-*erythro*	7.531 (16.0)	6.483	6.126 (5.1)	4.922	4.488 (5.9)	4.404 (4.2)
22-*threo*	7.838 (16.1)	6.737	6.137 (6.4)	4.806	4.280 (4.2)	4.041 (6.0)
22-*erythro*	7.837 (16.0)	6.739	6.095 (6.1)	4.852	4.391 (5.9)	4.238 (4.2)
23-*threo*	7.811 (16.0)	6.773	6.136 (6.4)	4.806	4.280 (4.2)	4.043 (5.6)
23-*erythro*	7.810 (16.0)	6.776	6.094 (5.1)	4.853	4.391 (5.8)	4.239 (4.2)

[a] In ppm. Coupling constants are listed in parentheses and are measured to within ±0.05 Hz. Proton designations are based on standard lignin nomenclature (see figures and schemes). The α-ester models **3** and **4** were recorded in acetone-d_6 at 300 K with materials that had been freeze-dried from D_2O.

1:1 ratios. Purification by silica gel chromatography subsequent to acetylation and deacetylation gave enriched threo fractions of **14–17**. Unfortunately, the deacetylation reactions to afford models **14** and **15** proceeded with some difficulty, with yields averaging near 62%.

The acetylated threo and erythro isomers of phenolate esters **22** and **23** (Scheme II) were prepared by similar but separate pathways starting with enriched threo and erythro precursors. For the threo isomers, 1-[4-(benzyloxy)-3-methoxyphenyl]-2-(2-methoxyphenoxy)propane-1,3-diol (**18**; 70% threo), the standard intermediate for the synthesis of 1-*threo* (Ralph and Helm, 1991), was acetylated and subsequently debenzylated. Purification of the debenzylation product by silica gel chromatography provided **19**-*threo* in 92% purity, the remaining 8% being the erythro isomer. Acylation of the phenolic hydroxyl with the appropriate 4-acetoxycinnamoyl chloride gave **22**-*threo* and **23**-*threo* in good yields.

The erythro isomers of **22** and **23** were prepared starting from the *erythro*-ethyl 3-[4-(benzyloxy)-3-methoxyphenyl]-3-hydroxy-2-(2-methoxyphenoxy)propionate (**20**-*erythro*; Nakatsubo et al., 1975). Reduction ($LiAlH_4$) and acetylation gave the crude *erythro*-benzyl ether, which was debenzylated to afford **21**-*erythro*. Acylation of the phenolic hydroxyl with the appropriate 4-acetoxycinnamoyl chloride gave the erythro isomers **22** and **23**.

Spectroscopic Characterization. The ^{1}H NMR data for the final products are shown in Table I. The assignment process was the same as described for the α-ethers (Helm and Ralph, 1992b). The $J_{\gamma,\beta}$ coupling constants are in the range 3.5–6.2 Hz, with the upfield threo γ-proton (γ_1) always having the higher value relative to γ_2 ($J_{\gamma1,\beta} > J_{\gamma2,\beta}$), while the opposite is true of the erythro isomers ($J_{\gamma1,\beta} < J_{\gamma2,\beta}$). This same general trend was also observed in the α-ethers (Helm and Ralph, 1992b). These differences are a manifestation of the threo/erythro stereochemistry which provides averaged rotameric distributions for each proton and thus different coupling constants. Labeling studies and the preparation of optically active lignin models would make possible unambiguous *pro-R* and *pro-S* assignments for these γ-protons. Two other trends in the ^{1}H NMR data were (A) the threo β-protons resonate slightly upfield of their erythro isomers and (B) the $J_{\alpha,\beta}$ values of the threo isomers were greater than those of their erythro counterparts.

The ^{13}C NMR data are shown in Tables II and III. The threo β-carbons of all compounds prepared are shifted slightly farther downfield (ca. 1 ppm) than those of the erythro isomers. The γ-esters (**14** and **15**) are readily differentiated from the α-esters (**3** and **4**) by the downfield shift exhibited by the carbon at the site of cinnamoylation. For example, the α-ester 4-erythro exhibits α and γ chemical shifts of 75.09 and 61.48 ppm, respectively (Table II), whereas its γ-ester isomer (**15**-*erythro*) has shifts of 73.26 and 63.94 ppm for these two carbons. These differences disappear upon acetylation (Table III) as both hydroxyls are acylated (compare **6**-*erythro* and **17**-*erythro*, the acetates of **4**- and **15**-*erythro*).

Even with only three aryl rings, the aromatic region of the ^{1}H spectra became quite complicated. At times the assignment of multiplets can be difficult due to overlapping peaks, and additional experiments are often necessary to aid in the assignment process. One of the simpler experiments which can help in assigning multiplets is the long-range or delayed COSY experiment (Bax and Freeman, 1981). Cross peaks due to long-range couplings with values less than the observable line width of the spectrometer are possible (Derome, 1987). This technique has been applied to the compounds prepared in this study to help assign the B-ring protons and consequently (via the one-bond C–H HMQC experiment) the B-ring carbons (Tables II and III). The results of such an experiment are exemplified in Figure 1 for **4**-*erythro*.

A delay of 400 ms was deemed the most effective at bringing out the long-range couplings needed to assign some of the more complex multiplets. The methoxyl protons of each of the three rings correlate with the adjacent protons at the 2-position (Figure 1a). A weaker correlation exists between the B-5 proton and the β-proton as shown in Figure 1b. The remaining B-ring protons can be assigned by reviewing the cross peaks present in standard COSY experiments as well as the long-range variant (Figure 1c). A-ring proton correlations to the α-proton facilitate their assignments as do the correlations between the F-7 proton and the F-ring protons.

General. Lam et al. (1992b) have recently reported that the amount of *p*-coumaric acid within the cell walls of wheat and phalaris increases with wall maturity. Since most of the *p*-coumaric acid present in several species has been found to be bound to lignin via ester-only linkages

Table II. ^{13}C NMR Data for the Model Trimer Esters[a,b]

	α-esters				γ-esters			
carbon	3-*threo*	3-*erythro*	4-*threo*	4-*erythro*	14-*threo*	14-*erythro*	15-*threo*	15-*erythro*
α	75.88	75.08	75.87	75.09	73.95	73.28	73.94	73.26
β	85.20	84.09	85.13	84.02	84.52	83.54	84.48	83.51
γ	61.76	61.49	61.74	61.48	64.18	63.98	64.16	63.94
A-1	130.16	129.64	130.15	129.63	133.16	133.59	133.16	133.63
A-2	112.04	112.57	112.04	112.60	111.46	111.20	111.46	111.21
A-3	148.18	147.99	148.19	147.98	148.09	148.02	148.08	148.05
A-4	147.48	147.35	147.50	147.36	147.02	146.72	147.01	146.74
A-5	115.55	115.21	115.59	115.22	115.34	115.24	115.33	115.25
A-6	121.23	121.79	121.20	121.80	120.56	120.32	120.56	120.31
B-1	123.24	123.31	123.21	123.30	123.55	123.51	123.54	123.49
B-2	113.58	113.67	113.54	113.66	113.59	113.64	113.60	113.66
B-3	151.87	151.86	151.82	151.84	151.79	152.02	151.80	152.04
B-4	149.82	149.22	149.78	149.19	149.36	148.86	149.36	148.86
B-5	119.36	119.18	119.25	119.10	119.42	119.67	119.39	119.63
B-6	121.78	121.75	121.76	121.76	121.81	121.71	121.81	121.73
C/F-1	127.04	126.97	127.44	127.40	126.89	126.91	127.35	127.37
C/F-2	130.91	130.96	111.26	111.29	130.94	130.88	111.37	111.29
C/F-3	116.64	116.67	148.70	148.71	116.66	116.64	148.71	148.70
C/F-4	160.48	160.59	150.02	150.09	160.63	160.52	150.09	150.04
C/F-5	116.64	116.67	116.03	116.04	116.66	116.64	116.05	116.03
C/F-6	130.91	130.96	123.91	123.99	130.94	130.88	123.90	123.86
C/F-7	145.39	145.65	145.77	146.02	145.57	145.39	145.91	145.74
C/F-8	115.81	115.62	115.99	115.83	115.22	115.34	115.47	115.59
C/F-9	166.51	166.32	166.55	166.34	167.15	167.28	167.14	167.28

[a] Values were determined in acetone-d_6 at 300 K with the central solvent peak as internal reference (29.80 ppm). [b] Chemical shift assignments are based on one- and two-dimensional NMR experiments, and stereochemical designations are based on the chemical shifts of the γ-protons. Numbering is based on standard lignin nomenclature (see figures and schemes).

Table III. ^{13}C NMR Data for the Model Trimer Ester Peracetates[a]

	α-esters				γ-esters				phenolate esters			
carbon	5-*threo*	5-*erythro*	6-*threo*	6-*erythro*	16-*threo*	16-*erythro*	17-*threo*	17-*erythro*	22-*threo*	22-*erythro*	23-*threo*	23-*erythro*
α	75.81	74.96	75.82	74.97	75.54	74.71	75.57	74.70	75.40	74.60	75.40	74.60
β	80.87	80.44	80.88	80.39	80.89	80.52	80.88	80.50	80.70	80.32	80.70	80.32
γ	63.70	63.16	63.70	63.16	63.94	63.33	63.96	63.33	63.60	63.00	63.61	63.00
A-1	136.74	136.63	136.77	136.61	136.71	136.73	136.69	136.73	136.78	136.78	136.77	136.77
A-2	112.71	112.79	112.72	112.79	112.70	112.74	112.69	112.74	112.70	112.77	112.70	112.77
A-3	152.24	152.14	152.24	152.14	152.22	152.11	152.23	152.12	152.30	152.19	152.30	152.19
A-4	140.95	140.83	140.95	140.83	140.91	140.77	140.92	140.79	140.83	140.71	140.83	140.70
A-5	123.61	123.40	123.61	123.41	123.59	123.37	123.60	123.38	123.64	123.45	123.64	123.44
A-6	120.31	120.45	120.30	120.43	120.32	120.44	120.32	120.44	120.34	120.45	120.34	120.45
B-1	123.78	124.08	123.77	124.08	123.81	124.10	123.80	124.41	123.77	124.06	123.77	124.06
B-2	113.66	113.80	113.65	113.79	113.73	113.76	113.73	113.77	113.74	113.79	113.75	113.79
B-3	151.89	152.06	151.88	152.05	151.90	152.09	151.89	152.10	151.84	152.06	151.84	152.06
B-4	149.12	148.39	149.13	148.37	149.12	148.30	149.10	148.30	149.08	148.27	149.08	148.27
B-5	119.33	119.89	119.30	119.85	119.36	119.99	119.33	119.97	119.23	119.82	119.23	119.82
B-6	121.65	121.68	121.65	121.68	121.69	121.64	121.69	121.65	121.68	121.64	121.68	121.64
C/F-1	132.82	132.82	134.15	134.13	132.81	132.78	134.13	134.11	132.72	132.73	134.04	134.04
C/F-2	130.22	130.28	112.42	112.46	130.25	130.21	112.45	112.44	130.44	130.44	112.61	112.60
C/F-3	123.21	123.24	152.67	152.69	123.21	123.20	152.68	152.67	123.31	123.31	152.76	152.76
C/F-4	153.52	153.58	142.76	142.82	153.52	153.49	142.76	142.74	153.74	153.74	142.98	142.97
C/F-5	123.21	123.24	124.12	124.14	123.21	123.20	124.12	124.41	123.31	123.31	124.20	124.20
C/F-6	130.22	130.28	122.27	122.35	130.25	130.21	122.30	122.25	130.44	130.44	122.52	122.52
C/F-7	144.91	145.12	145.36	145.57	144.75	144.71	145.20	145.16	146.05	146.02	146.46	146.44
C/F-8	118.76	118.67	118.85	118.75	118.57	118.54	118.64	118.63	117.92	117.95	118.03	118.06
C/F-9	165.90	165.79	165.96	165.89	166.61	166.60	166.68	166.67	164.91	164.95	164.97	165.00

[a] See Table II for reference and assignment details.

(Smith, 1955; Shimada et al., 1971; Scalbert et al., 1985; Lam et al., 1992a), it has been proposed (Lam et al., 1992b) that this linkage pattern results from the addition, subsequent to lignification, to the quinone methide of lignin β-*O*-4 intermediates. This is in contrast to efforts of Higuchi's group (Shimada et al., 1971; Nakamura and Higuchi, 1978), where evidence for γ-esterification of *p*-coumaric acid to lignin was presented. In addition, our studies with compounds **3** and **4** reveal that when the A-ring phenolic hydroxyl is free, there is a marked instability for α-esters to aqueous environments. Thus, if hydroxycinnamoyl α-esters are present in grass cell walls, formation would presumably have to occur during lignification so as to subsequently stabilize the linkage by free-radical condensation of the A-ring hydroxyl into the lignin macromolecule.

Esterification at the γ-position of lignin could result either from an enzyme-mediated acylation of the lignin polymers or from the direct participation of preformed coniferyl *p*-coumarate in lignification (Ralph and Helm, 1993). Indeed, DHP studies with coniferyl *p*-coumarate have indicated direct incorporation of the *p*-coumarate

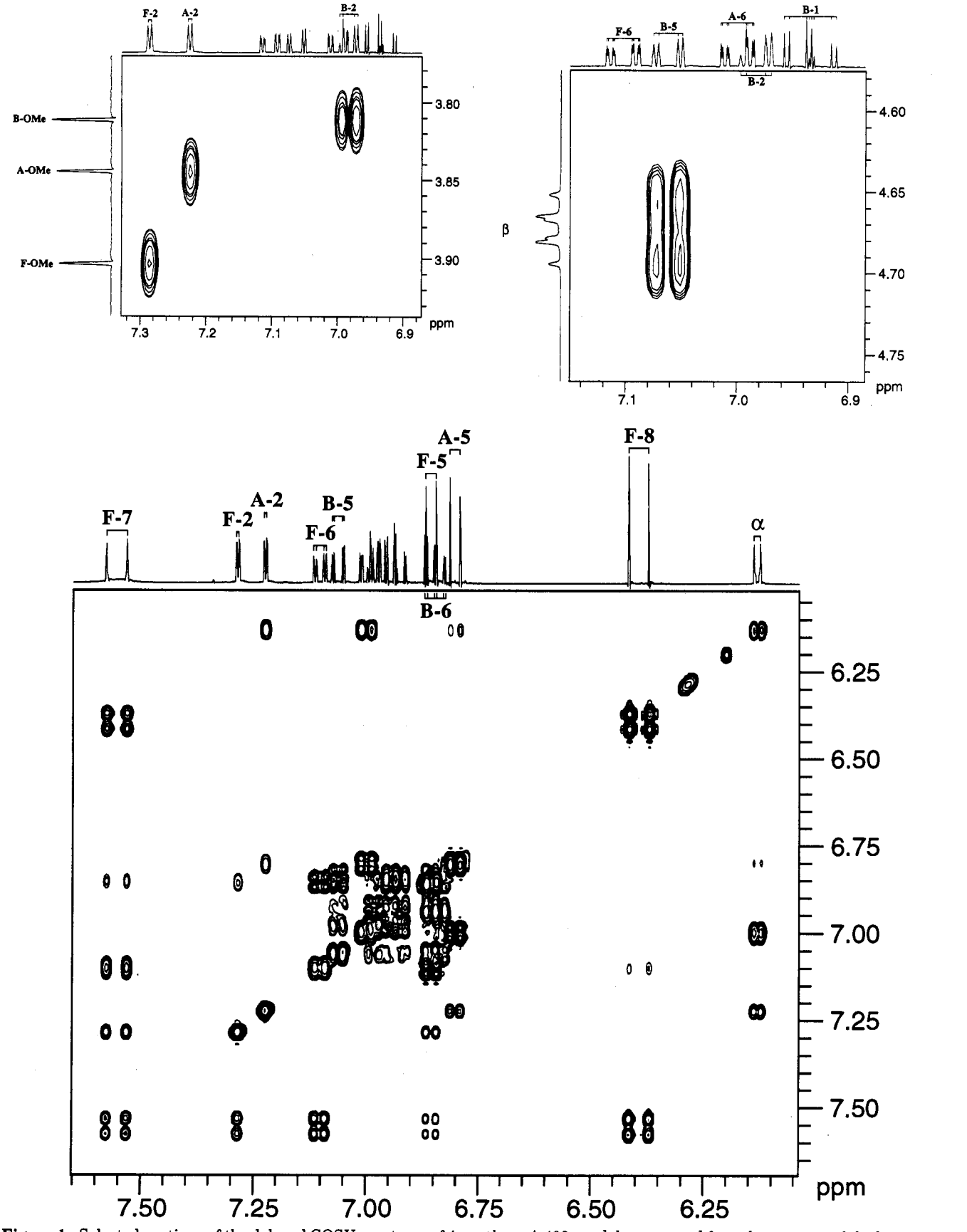


Figure 1. Selected portions of the delayed COSY spectrum of 4-*erythro*. A 400-ms delay was used for enhancement of the long-range correlations, and the processed spectrum was not symmetrized. (a, top left) Correlations between the aryl methoxyls and the adjacent protons at the 2-position; (b, top right) correlation between the B-5 proton and the β-proton; (c, bottom) correlations in the aromatic region.

moiety into etherified structures (Nakamura and Higuchi, 1978). Efforts are currently underway in this laboratory to examine the modes by which coniferyl *p*-coumarate is incorporated into synthetic lignin dehydrogenation polymers as well as the location of the esterified *p*-coumaric acid in native lignin isolates.

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