Facile Synthesis of 4-Hydroxycinnamyl *p*-Coumarates

Fachuang Lu and John Ralph*

U.S. Dairy Forage Research Center, Agricultural Research Service, U.S. Department of Agriculture, and Department of Forestry, University of Wisconsin—Madison, Madison, Wisconsin 53706

Coniferyl *p*-coumarate (4-hydroxy-3-methoxycinnamyl 4-hydroxycinnamate) and sinapyl *p*-coumarate (3,5-dimethoxy-4-hydroxycinnamyl 4-hydroxycinnamate) were synthesized in high overall yield. In this improved method acetate was used as the phenol protecting group instead of 2,4-dinitrophenyl ether; selective deacetylation, without cinnamate ester hydrolysis, was accomplished with neat pyrrolidine.

Keywords: *Coniferyl p-coumarate; sinapyl p-coumarate; lignin; acylation; grasses; radical coupling; deacetylation*

INTRODUCTION

Cell walls of grasses contain substantial amounts of *p*-coumarates on their lignins. Recent NMR studies on maize and other grasses have demonstrated that exclusively the primary γ-positions of lignin side chains are acylated by *p*-coumaric acid (Ralph et al., 1994; Crestini and Argyropoulos, 1997). Coniferyl and sinapyl *p*-coumarates **6** are logical precursors of these γ-ester structures (Nakamura and Higuchi, 1976, 1978a,b; Ralph et al., 1994). Although functional roles for related ferulates have been adduced (Ralph et al., 1992, 1998), the role of *p*-coumarates in cell wall structure and development is still not well understood. An interesting observation is that the *p*-coumarate moiety does not incorporate (via the radical coupling mechanisms that characterize lignification) into lignin and remains as a terminal unit with a free phenolic group. However, when synthetic lignins are made with coniferyl *p*-coumarate and coniferyl alcohol, the *p*-coumarate moieties of the esters appear to be incorporated into lignin via radical pathways (Nakamura and Higuchi, 1978b). The reasons for such a difference between natural lignin and synthetic lignins are only now being revealed. Recent studies indicated that *p*-coumarates may function as radical transfer carriers to help sinapyl alcohol incorporate into lignin when the wall peroxidases have a low reactivity with sinapyl alcohol directly (Takahama et al., 1996; Hatfield et al., 1997).

Coniferyl and sinapyl *p*-coumarates are possible precursors of grass lignins and are required for basic studies into their role in lignification. They are traditionally synthesized by coupling of protected *p*-coumaroyl chloride and protected 4-hydroxycinnamyl alcohols (Nakamura and Higuchi, 1978a; Ralph et al., 1994; Grabber et al., 1996). Many protecting groups have been used for protecting the phenolic hydroxyl of 4-hydroxycinnamyl alcohols; 2,4-dinitrophenyl ether was considered the best one for this purpose, allowing coniferyl *p*-coumarate to be prepared in moderate yield (Nakamura and Higuchi, 1978a). Here we report a facile way to prepare 4-hydroxycinnamyl *p*-coumarates in 80−85% overall yields from 4-hydroxycinnamaldehydes. The new method protects both the alcohol and the acid moieties using acetyl protecting groups that are not normally considered due to the impression that they could not be selectively removed (without cleaving the coumarate ester). Deprotection is consequently a single step.

* Address correspondence to this author at the U.S. Dairy Forage Research Center, USDA-ARS, 1925 Linden Dr. W., Madison, WI 53706-1108 [telephone (608) 264-5407; fax (608) 264-5147; e-mail jralph@facstaff.wisc.edu].

EXPERIMENTAL PROCEDURES

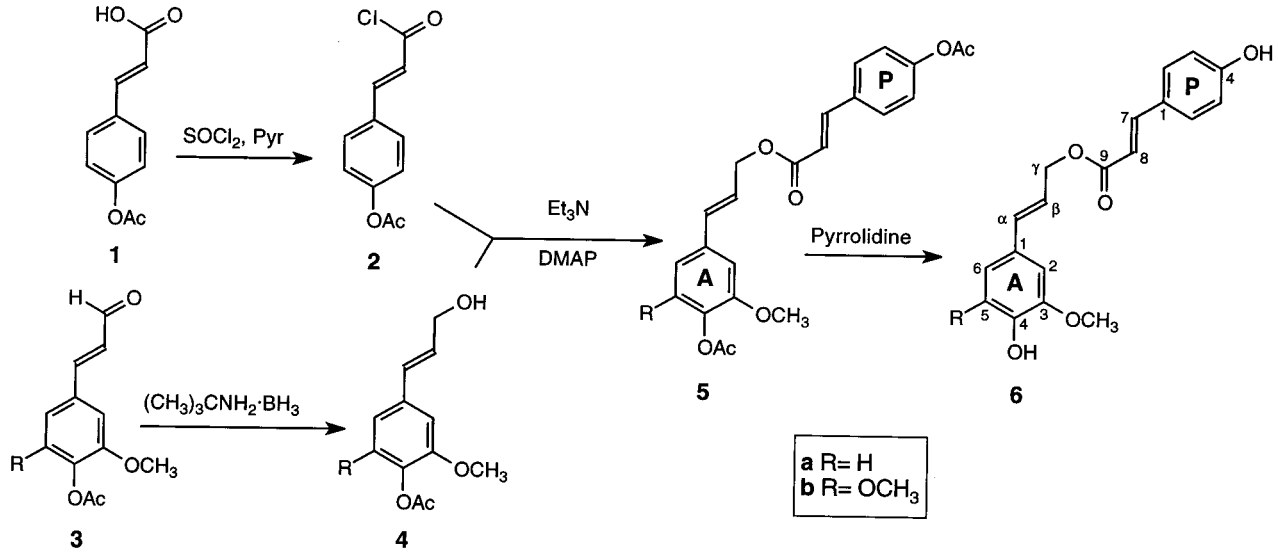
General Methods. Melting points were measured on an Electrothermal (Southend, U.K.) digital mp apparatus and were uncorrected. Evaporations were conducted under reduced pressures at temperatures <40 °C. Further removal of organic solvents, as well as drying of residues, was accomplished under higher vacuum (100−200 mTorr) at room temperature. Flash chromatography was performed on FLASH 40M cartridges (Biotage, Dyax Corp., Charlottesville, VA) using a UA-6 UV−vis detector (ISCO, Lincoln, NE). Solid phase extraction was carried out with 3 mL LC-Si tubes (Supelco, Bellefonte, PA) using $CHCl_3$/EtOAc (19:1) as solvent. NMR spectra of samples in acetone-d_6 were run at 300 K on a Bruker AMX-360 MHz narrow-bore instrument (Bruker Instruments, Billerica, MA) fitted with a 5 mm four-nucleus (QNP) probe with normal geometry (proton coil further from the sample). The central solvent signals were used as reference (^{1}H, 2.04 ppm; ^{13}C, 29.80 ppm). Fully authenticated assignments were made by the usual complement of 1D and 2D methods, using standard Bruker pulse programs. Petroleum ether refers to the boiling range 40−60 °C. Coniferaldehyde, sinapaldehyde, and acetylation reagents were from Aldrich (Milwaukee, WI) and used as supplied. Solvents (AR grade) were from Mallinckrodt (Paris, KY).

Precursors. *4-Acetoxycinnamoyl Chloride (**2**).* Chloride **2** was prepared (white crystals in 96% yield) from *p*-coumaric acid by acetylation followed by chlorination using $SOCl_2$ according to a previous method (Helm et al., 1992).

*4-Acetoxycinnamyl Alcohols **4a**,**b**.* 4-Acetoxycinnamaldehydes **3a**,**b** were prepared in 94−96% yield by acetylation of the corresponding 4-hydroxycinnamaldehydes with Ac_2O/pyridine and then reduced with borane/*tert*-butylamine complex to give the corresponding alcohols **4a**,**b** as follows. The 4-acetoxycinnamaldehyde was dissolved in methylene chloride to which borane/*tert*-butylamine complex (1.5 equiv) was added. The mixture was stirred at room temperature for 2 h, when TLC showed that the starting material had disappeared completely. The solvent was evaporated at 40 °C under

S0021-8561(98)00440-3 CCC: $15.00
Published on Web 07/24/1998

Scheme 1. Synthetic Scheme for Coniferyl and Sinapyl *p*-Coumarates



reduced pressure. The residue was hydrolyzed with 0.5 M H_2SO_4 in ethanol/water (1:1) for 1.5 h. Most of the ethanol was removed by evaporation, and the product was extracted with ethyl acetate. The ethyl acetate solution was washed with saturated NH_4Cl and dried over $MgSO_4$. Evaporation of the ethyl acetate gave the product as a light yellow syrup. **4a**: pale yellow oil (96% yield); ^{1}H NMR (acetone-d_6) δ 2.31 (3H, s, OAc), 3.83 (3H, s, OAc), 3.90 (1H, t, J = 5.5 Hz, γ-OH), 4.22 (2H, dt, J = 5.5, 1.7 Hz, γ's), 6.38 (1H, dt, J = 15.9, 5.2 Hz, β), 6.58 (1H, dt, J = 15.9, 1.7 Hz, α), 6.97 (2H, m, A5/6), 7.15 (s, 1H, A2); ^{13}C NMR δ 20.5 (OAc), 56.2 (OMe), 63.1 (γ), 110.9 (A2), 119.5 (A6), 123.6 (A5), 129.3 (α), 131.4 (β), 137.2 (A1), 140.2 (A4), 152.3 (A3), 169.0 (OAc). **4b**: (94.5% yield); crystallization from methylene chloride/petroleum ether afforded white plate crystals; mp 108.6−109.8 °C [lit. (Daubresse et al., 1994) 85 °C]; ^{1}H NMR δ 2.21 (3H, s, OAc), 3.80 (6H, s, OMe's), 3.89 (1H, t, J = 5.5 Hz, γ-OH), 4.23 (2H, td, J = 5.5, 1.6 Hz, γ's), 6.40 (1H, dt, J = 15.9, 5.0 Hz, β), 6.57 (1H, dt, J = 15.9, 1.6 Hz, α), 6.78 (2H, s, A2/6); ^{13}C NMR δ 20.2 (OAc), 56.4 (A3/5-OMe's), 63.0 (γ), 103.8 (A2/6), 129.1 (A1), 129.7 (β), 131.43 (α), 136.6 (A4), 153.3 (A3/5), 168.6 (OAc).

4-Acetoxycinnamyl *p*-Coumarates 5a,b. Coupling of 4-acetoxycinnamoyl chloride (**2**) with 4-acetoxycinnamyl alcohols **4a**,**b** was efficiently carried out using 4-(dimethylamino)-pyridine (DMAP). Thus, **4a** (2.3 g, 10.36 mmol) and chloride **2** (2.6 g, 11.4 mmol) were dissolved in dry CH_2Cl_2 (120 mL) to which DMAP (316 mg, 0.25 equiv) and Et_3N (1.24 mL, 0.85 equiv) were added. The mixture was stirred for ~2 h, when TLC [$CHCl_3$/EtOAc (5:1)] showed the starting material **4a** was converted into a faster moving compound. The solution was diluted with CH_2Cl_2 and washed successively with aqueous 3% HCl and saturated NH_4Cl. Drying over $MgSO_4$, evaporation, and purification by flash chromatography [$CHCl_3$/EtOAc (19:1)] gave **5a** (4.09 g, 94%) as a pale yellow oil. **5a**: ^{1}H NMR δ 2.22 (3H, s, A-OAc), 2.27 (3H, s, P-OAc), 3.84 (3H, s, A3-OMe), 4.85 (2H, dd, J = 6.3, 1.3 Hz, γ's), 6.44 (1H, dt, J = 15.9, 6.3 Hz, β), 6.57 (1H, d, J = 16.0 Hz, P8), 6.75 (1H, dt, J = 15.9, 1.3 Hz, α), 7.00 (1H, d, J = 8.2 Hz, A5), 7.05 (1H, dd, J = 8.2, 1.7 Hz, A6), 7.20 (2H, m, P3/5), 7.24 (1H, d, J = 1.7 Hz, A2), 7.72 (1H, d, J = 16.0 Hz, P7), 7.74 (2H, m, P2/6); ^{13}C NMR δ 20.5 (A-OAc), 20.9 (P−OAc), 56.2 (OMe), 65.3 (γ), 111.17 (A2), 118.9 (P8), 120.0 (A6), 123.3 (P3/5), 123.8 (A5), 124.9 (α), 130.2 (P2/6), 132.9 (P1), 133.9 (β), 136.3 (A1), 140.8 (A4), 144.6 P7), 152.5 (A3), 153.5 (P4), 166.7 (P9), 168.9 (A-OAc), 169.4 (P-OAc); FAB MS, found Na + M^+, 433.0, $NaC_{23}H_{22}O_7$ requires M, 433.1.

5b was prepared in 92% yield as a pale yellow oil in the same way as described for **5a**. Crystallization from acetone/petroleum ether afforded white crystalline **5b** in 80% yield. **5b**: mp 100.8−102.0 °C; ^{1}H NMR δ 2.22 (3H, s, A-OAc), 2.27 (3H, s, P-OAc), 3.82 (6H, s, A3-OMe), 4.85 (2H, dd, J = 6.3, 1.3 Hz, γ's), 6.44 (1H, dt, J = 15.9, 6.3 Hz, β), 6.57 (1H, d, J = 16.0 Hz, P8), 6.72 (1H, dt, J = 15.9, 1.3 Hz, α), 6.86 (2H, s, A2/6), 7.20 (2H, m, P3/5), 7.72 (1H, d, J = 16.0 Hz, P7), 7.74 (2H, m, P2/6); ^{13}C NMR δ 20.2 (A-OAc), 20.9 (P-OAc), 56.5 (OMe), 65.3 (γ), 104.2 (A2/6), 118.9 (P8), 123.3 (P3/5), 125.0 (β), 129.7 (A1), 130.2 (P2/6), 132.9 (P1), 134.2 (α), 135.7 (A1), 144.6 (P7), 153.4 (A3/5), 153.5 (P4), 166.7 (P9), 168.5 (A-OAc), 169.4 (P-OAc); FAB MS, found Na + M^+, 463.1, $NaC_{24}H_{24}O_8$ requires M, 463.1.

4-Hydroxycinnamyl *p*-Coumarates 6a,b. For compound **6a**, 4-acetoxy-3-methoxycinnamyl 4-acetoxy-*p*-coumarate (**5a**; 80 mg, 0.195 mmol) was dissolved in pyrrolidine (1 mL). Once dissolution was complete, the pyrrolidine solution was diluted with 50 mL of ethyl acetate and washed with 1 M H_2SO_4 (3 × 20 mL) and saturated NH_4Cl (2 × 20 mL). After drying over $MgSO_4$ and evaporation, the resulting syrup was submitted to solid phase extraction [$CHCl_3$/EtOAc (19:1)] to afford **6a** (59 mg, 93%) as a pale yellow syrup. **6a**: ^{1}H NMR δ 3.86 (6H, s, A3/5-OMe's), 4.79 (2H, dd, J = 6.5, 1.2 Hz, γ's), 6.25 (1H, dt, J = 15.9, 6.5 Hz, β), 6.38 (1H, d, J = 16.0 Hz, P8), 6.65 (1H, dt, J = 15.9, 1.2 Hz, α), 6.80 (1H, d, J = 8.1 Hz, A5), 6.90 (2H, m, P3/5), 6.92 (1H, dd, J = 8.1, 1.9 Hz, A6), 7.11 (1H, d, J = 1.9 Hz, A2), 7.54 (2H, m, P3/5), 7.64 (1H, d, J = 16.0 Hz, P7); ^{13}C NMR δ 56.2 (A-OMe), 65.5 (γ), 110.1 (A2), 115.5 (P8), 115.8 (A5), 116.7 (P3/5), 121.2 (A6), 121.7 (β), 127.0 (P1), 129.4 (A1), 130.9 (P2/6), 134.9 (α), 145.5 (P7), 147.7 (A4), 148.7 (A3/5), 160.5 (P4), 167.4 (P9).

Compound **6b** was prepared as a clear syrup in 94% yield from **5b** according to the procedure described for **6a**. **6b**: ^{1}H NMR δ 3.84 (6H, s, A3/5-OMe's), 4.78 (2H, dd, J = 6.4, 1.3 Hz, γ's), 6.28, 1H, dt, J = 15.8, 6.4 Hz, β), 6.38 (1H, d, J = 16.0 Hz, P8), 6.63 (1H, dt, J = 16.0, 1.3 Hz, α), 6.80 (2H, s, A2/6), 6.89 (2H, m, P3/5), 7.55 (2H, m, P2/6), 7.60 (1H, d, J = 16.0 Hz, P7); ^{13}C NMR δ 56.6 (A3/5-OMe's), 65.4 (γ), 105.1 (A2/6), 115.5 (P8), 116.7 (P3/5), 122.1 (β), 127.0 (P1), 128.2 (A1), 130.9 (P2/6), 135.1 (α), 137.2 (A4), 145.5 (P7), 148.8 (A3/5), 160.6 (P4), 167.2 (P9).

RESULTS AND DISCUSSION

Synthetic strategies for coniferyl *p*-coumarate have been discussed (Nakamura and Higuchi, 1978a). The ideal way to prepare the target compound would be direct coupling of the acid and alcohol moieties without using any protecting groups. Previous attempts using this method failed for the obvious regioselectivity reasons; to our knowledge, the use of enzymes (lipases, for example) for this esterification has not been examined. Therefore, the phenolic hydroxyls on the precursor acid and alcohol have to be protected prior to esterification.

The benzyl ether has been avoided because its deprotection conditions, catalytic hydrogenolysis, concomitantly saturate the double bonds on both acid and alcohol moieties. Esters are not normally considered as protecting groups due to the possibility of simultaneous hydrolysis of the desired ester linkage during deprotection. 4-Hydroxycinnamyl *p*-coumarates are sensitive to both basic and acidic conditions; even acetic acid at room temperature caused some hydrolysis of these esters. The most successful strategy to prepare coniferyl *p*-coumarate has been to start with coniferyl alcohol and use 2,4-dinitrophenyl ether for protection of one or both phenols because of its relatively mild removal by nucleophilic agents (Nakamura and Higuchi, 1978a; Grabber et al., 1996).

Pyrrolidine was found to selectively deacetylate phenolic acetates over aliphatic acetates and has been used for determination of phenolic hydroxyl groups in lignins (Mansson, 1982, 1983). We have used pyrrolidine for preparation of 4-hydroxy-3-methoxycinnamyl acetate in high yield from coniferyl diacetate (Lu, 1996, unpublished results). However, coniferyl alcohol cannot be used as the starting material if acetate is chosen as the protecting group because selective acetylation of the phenolic hydroxyl over the aliphatic one, although possible, is not straightforward. Fortunately, with the recent commercial availability of 4-hydroxycinnamaldehydes, 4-acetoxy-3-methoxycinnamyl alcohol (the phenolic acetate of coniferyl alcohol) is readily prepared. Starting with 4-hydroxycinnamaldehydes, 4-hydroxycinnamyl *p*-coumarates can now easily be prepared in high overall yields (Scheme 1).

4-Acetoxycinnamaldehydes **3a**,**b** were obtained from coniferaldehyde/sinapaldehyde by acetylation with acetic anhydride/pyridine in 94−96% yield. Regioselective 1,2-reduction using borane/*tert*-butylamine complex in methylene chloride gave rise to 4-acetoxycinnamyl alcohols **4a**,**b** in high yield (95%) without dihydro products from 1,4-reduction. 4-Acetoxycinnamyl alcohol **4b** was crystallized from methylene chloride/petroleum ether as white plates with a melting point of 108−109 °C, higher than previously described (Daubresse et al., 1994). From 4-hydroxycinnamic acid, 4-acetoxycinnamoyl chloride (2) was prepared as white crystals in 96% yield by acetylation and chlorination ($SOCl_2$) according to a previously described method (Helm et al., 1992). The coupling conditions (DMAP in Et_3N) used for ester formation were the same as used previously (Grabber et al., 1996). Deacetylation of **5a**,**b** is the key to accomplish the efficient syntheses of the target compounds **6a**,**b**. Preliminary trials for deacetylation of **5a** using pyrrolidine with dioxane as cosolvent, similar to conditions for determination of phenolic hydroxyls in lignins (Mansson, 1982, 1983), gave **6a** in only 50% yield due to hydrolysis. However, deacetylation in neat pyrrolidine produced **6a**,**b** in >90% yield each, after purification through solid phase extraction (see Experimental Procedures).

The resulting syntheses of 4-hydroxycinnamyl *p*-coumarates are simpler and more efficient than previous methods. The primary advantages of the present method are (1) the use of acetates as protecting groups and simple selective deacetylation techniques and (2) the use of commercial 4-hydroxycinnamaldehydes as starting materials and their simple pathway into suitably protected 4-hydroxycinnamyl alcohols.

LITERATURE CITED

Crestini, C.; Argyropoulos, D. S. Structural analysis of wheat straw lignin by quantitative ^{31}P and 2D NMR spectroscopy. The occurrence of ester bonds and α-O-4. *J. Agric. Food Chem.* **1997**, *45*, 1212−1219.

Daubresse, N.; Francesch, C.; Mhamdi, F.; Rolando, C. A mild synthesis of coumaryl, coniferyl, sinapyl aldehydes and alcohols. *Synthesis* **1994**, 369−371.

Grabber, J. H.; Quideau, S.; Ralph, J. *p*-Coumaroylated syringyl units in maize lignin; implications for *β*-ether cleavage by thioacidolysis. *Phytochemistry* **1996**, *43*, 1189−1194.

Hatfield, R. D.; Grabber, J.; Ralph, J. A potential role of sinapyl *p*-coumarate in grass lignin formation. *Plant Physiol. (Suppl.)* **1997**, *114* (346), 86.

Helm, R. F.; Ralph, J.; Hatfield, R. D. Synthesis of feruloylated and *p*-coumaroylated methyl glycosides. *Carbohydr. Res.* **1992**, *229*, 183−194.

Mansson, P. Selective deacetylation of aromatic acetates by aminolysis. *Tetrahedron Lett.* **1982**, *23*, 1845−1846.

Mansson, P. Quantitative determination of phenolic and total hydroxyl groups in lignins. *Holzforschung* **1983**, *37*, 143−146.

Nakamura, Y.; Higuchi, T. Ester linkage of *p*-coumaric acid in bamboo lignin. *Holzforschung* **1976**, *30*, 187−191.

Nakamura, Y.; Higuchi, T. Ester linkage of *p*-coumaric acid in bamboo lignin. II. Syntheses of coniferyl *p*-hydroxybenzoate and coniferyl *p*-coumarate as possible precursors of aromatic acid esters in lignin. *Cellul. Chem. Technol.* **1978a**, *12*, 199−208.

Nakamura, Y.; Higuchi, T. Ester linkage of *p*-coumaric acid in bamboo lignin. III. Dehydrogenative polymerization of coniferyl *p*-hydroxybenzoate and coniferyl *p*-coumarate. *Cellul. Chem. Technol.* **1978b**, *12*, 209−221.

Ralph, J.; Helm, R. F.; Quideau, S.; Hatfield, R. D. Lignin-feruloyl ester cross-links in grasses. Part 1. Incorporation of feruloyl esters into coniferyl alcohol dehydrogenation polymers. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2961−2969.

Ralph, J.; Hatfield, R. D.; Quideau, S.; Helm, R. F.; Grabber, J. H.; Jung, H.-J. G. Pathway of *p*-coumaric acid incorporation into maize lignin as revealed by NMR. *J. Am. Chem. Soc.* **1994**, *116*, 9448−9456.

Ralph, J.; Hatfield, R. D.; Grabber, J. H.; Jung, H. G.; Quideau, S.; Helm, R. F. Cell Wall Cross-linking in Grasses by Ferulates and Diferulates. In *Lignin and Lignan Biosynthesis*; Lewis, N. G., Sarkanen, S., Eds.; American Chemical Society: Washington, DC, 1998; pp 209−236.

Takahama, U.; Oniki, T.; Shimokawa, H. A possible mechanism for the oxidation of sinapyl alcohol by peroxidase-dependent reactions in the apoplast: enhancement of the oxidation by hydroxycinnamic acids and components of the apoplast. *Plant Cell Physiol.* **1996**, *37*, 499−504.

Received for review May 1, 1998. Revised manuscript received May 1, 1998. Accepted June 16, 1998. We gratefully acknowledge support through USDA-NRI Competitive Grant 96-02587 (Plant Growth and Development section).

JF980440Y