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Rapid Syntheses of Dehydrodiferulates via Biomimetic Radical Coupling Reactions of Ethyl Ferulate

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ABSTRACT: Dehydrodimerization of ferulates in grass cell walls provides a pathway toward cross-linking polysaccharide chains limiting the digestibility of carbohydrates by ruminant bacteria and in general affecting the utilization of grass as a renewable bioresource. Analysis of dehydrodiferulates (henceforth termed diferulates) in plant cell walls is useful in the evaluation of the quality of dairy forages as animal feeds. Therefore, there has been considerable demand for quantities of diferulates as standards for such analyses. Described here are syntheses of diferulates from ethyl ferulate via biomimetic radical coupling reactions using the copper(II)−tetramethylethylenediamine [CuCl(OH)−TMEDA] complex as oxidant or catalyst. Although CuCl(OH)−TMEDA oxidation of ethyl ferulate in acetonitrile produced mixtures composed of 8−*O*−4-, 8−5-, 8−8- (cyclic and noncyclic), and 5−5-coupled diferulates, a catalyzed oxidation using CuCl(OH)−TMEDA as catalyst and oxygen as an oxidant resulted in better overall yields of such diferulates. Flash chromatographic fractionation allowed isolation of 8−8- and 5−5-coupled diferulates. 8−5-Diferulate coeluted with 8−*O*−4-diferulate but was separated from it via crystallization; the 8−*O*−4 diferulate left in the mother solution was isolated by rechromatography following a simple tetrabutylammonium fluoride treatment that converted 8−5-diferulate to another useful diferulate, 8−5-(noncyclic) diferulate. Therefore, six of the nine (5−5, 8−*O*−4, 8−5-c, 8−5-nc, 8−5-dc, 8−8-c, 8−8-nc, 8−8-THF, 4−*O*−5) diferulic acids that have to date been found in the alkaline hydrolysates of plant cell walls can be readily synthesized by the CuCl(OH)−TMEDA catalyzed aerobic oxidative coupling reaction and subsequent saponification described here.

KEYWORDS: *copper(II)−tetramethylethylenediamine, diferulates, oxidative coupling, plant cell walls, synthesis, NMR*

INTRODUCTION

Ferulates, particularly in the form of polysaccharide ferulate esters, are widely found in the cell walls of forage plants, including within monocotyledons of the Poaceae and within dicotyledons included in the Amaranthaceae.[1] In monocots, ferulate acylates the primary hydroxyl at the C-5 position of α-L-arabinofuranosyl residues and on xylose side-chain residues of xyloglucans in bamboo, whereas in dicots, such as spinach and sugar beet, feruloylation occurs on arabinose or galactose side chains of pectic polysaccharides.[2]

Although ferulates exist in relatively low amounts in cell walls, they play very important roles not only for the biology of the wall but also for their structure. First, peroxidase-mediated oxidative coupling of ferulates cross-links polysaccharides, whereas analogous cross-coupling reactions between ferulates (or the resultant dehydrodiferulates) and monolignols or growing lignin polymers produce so-called lignin−ferulate−polysaccharide complexes.[3] Second, such cross-links have been shown to be responsible for the limited digestibility of grass forages by ruminant bacteria[4] and to contribute to the recalcitrance of grass cell walls to enzymatic hydrolysis by cellulases or xylanases[5] and have also been postulated to be responsible for controlling cell wall extensibility[6] and protection against pathogen invasion.[7] Finally, the ability of ferulates to cross-couple with monolignols and the finding of evidence for ferulate−monolignol cross-coupling in grasses[8] imply that ferulates and diferulates may act as nucleation sites for lignification.[9] Meanwhile, as important antioxidants in many human foods such as vegetables, fruits, and cereals, ferulates are proposed to exhibit a wide range of important biological and therapeutic properties including anti-inflammatory, antibacterial, antidiabetic, anticarcinogenic, antiaging, and neuroprotective effects.[10] In recent years, with increased interest in renewable bioresources as alternatives to fossil fuels for energy and materials production, lignocellulosic biomass sources, including grasses, are emerging as potential feedstocks for the production of biofuels and chemicals.[11]

Pretreatment processes become essential before biological conversion of lignocellulosic biomass to biofuels. Modification of grasses via manipulation of genes responsible for feruloylation of polysaccharides has been a focus in plant research because such modified plants potentially have cell walls with desirable properties, including better digestibility by ruminants, and are more easily processed as feedstocks that are saccharified and fermented for bioenergy and materials production.[12]

Therefore, studying the effects of ferulates and especially their dehydrodimers, the dehydrodiferulates (henceforth simply termed diferulates or ferulate dimers), which are markers for polysaccharide−polysaccharide cross-linking, on cell wall properties has become crucial. Such studies require ready accessibility to diferulates as reference compounds for qualitative and quantitative analysis of diferulates,[13] as well as

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model substrates for evaluating their potential as antioxidants.[14] However, some diferulates are not readily available by synthesis; for example, our original preparation of 8−*O*−4-diferulate involved as many as nine steps[3] and is therefore accessible only to skilled synthetic chemists. Cereal brans such as corn, wheat, and rice brans are good sources for isolating ferulic acid dimers. With demanding separation techniques including Sephadex LH-20 column fractionation and semi-preparative high-performance liquid chromatography (HPLC), as many as seven diferulic acids have been isolated with sufficient purity and in quantities (tens of milligrams) suitable for being used as standard compounds.[15] Electrochemical oxidative coupling of ferulate was reported[16] to produce isomeric diferulate mixtures containing 8−*O*−4-, 8−5-, and 8−8-diferulates (Figure 1). Each diferulate was isolated by flash chromatography. This approach seems to be a good way to synthesize diferulates, but the required device for electrochemical reactions and the limited amounts of products obtained prevent it from becoming a convenient and general protocol. Therefore, there remains a need to develop rapid and robust methods to produce diferulates in significant quantities without requiring particularly demanding separation skills and using readily accessible methods and instruments.

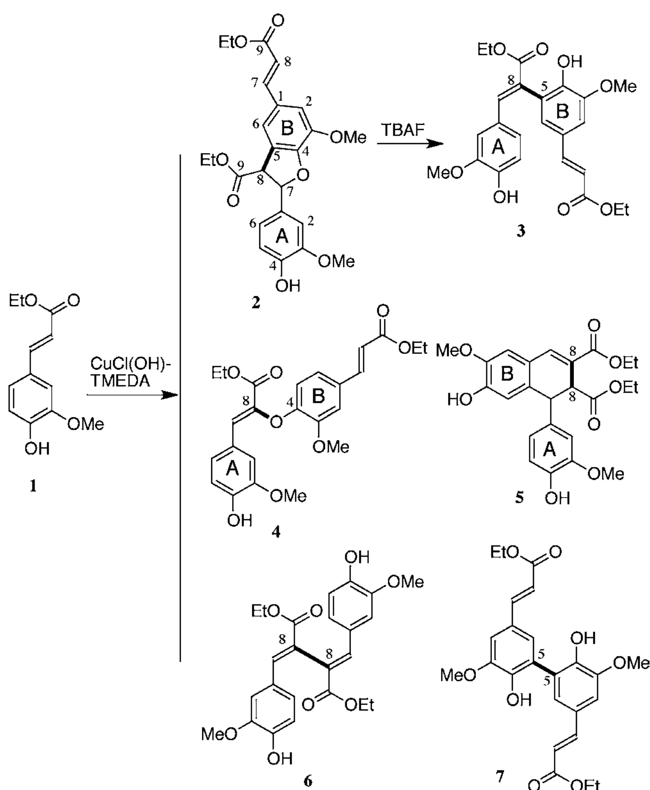

Figure 1. Radical coupling of ethyl ferulate **1** by CuCl(OH)−TMEDA (O_2) to produce the ferulate dehydrodimers **2**−**7**. Purification via simple flash chromatography is described in the text.

Our group aims to develop efficient and simple methods to make compounds of interest to aid our own research and that of others. In this study, we explored the possibility of using a Cu(II)−amine complex as a biomimetic oxidant or catalyst for oxidative coupling of ethyl ferulate to produce diferulates. Here we report on the rapid syntheses of dehydrodiferulates using the CuCl(OH)−tetramethylethylenediamine [CuCl(OH)−TMEDA] complex as either a stoichiometric oxidant or a catalyst for biomimetic oxidative coupling of ethyl ferulate to produce 8−*O*−4-, 8−5-, 8−8-, and 5−5-coupled diferulates (Figure 1). This biomimetic approach takes advantage of easy-to-perform coupling reactions (without requiring excessive levels of synthetic chemistry skill) and compound separation using simple flash chromatography to produce reasonable quantities (tens to hundreds of milligrams) of all the major diferulates found in plant cell walls.

■ MATERIALS AND METHODS

Materials. Ferulic acid was purchased from MP Biomedicals (Solon, OH, USA). Copper(I) chloride was from Alfa Aesar (Ward Hill, MA, USA), and tetramethylethylenediamine (TMEDA) was an Acros product (Fisher Scientific, Pittsburgh, PA, USA). CuCl(OH)−TMEDA was prepared in situ by adding an equimolar equivalent of TMEDA into copper(I) chloride suspended in acetonitrile. All solvents used were of analytical grade and obtained from Fisher. Silica gel flash chromatography was on a Biotage Isolera system (Biotage, Charlotte, NC, USA) using prepacked SNAP columns (10, 25, or 100 g of silica gel).

Ferulate Monomer. Ethyl ferulate was trivially prepared from ferulic acid, as described previously.[3] Thus, ferulic acid (25 g) was dissolved in absolute ethanol (200 mL), and acetyl chloride (15 mL) was added slowly. The solution was stirred gently for 2 days, after which the volatiles were removed by rotary evaporation at 40 °C. Addition of further ethanol and evaporation several times removed the residual HCl. The oily product was allowed to crystallize from EtOAc/cyclohexane to produce light yellow crystals (26.4 g, 90%).

Preparation of Dehydrodiferulates. *Method A [CuCl(OH)−TMEDA Oxidation].* An molar equivalent of TMEDA (1.16 g, 10 mmol) was added into anhydrous CuCl (1.0 g, 10 mmol) suspended in acetonitrile (200 mL) in a 500 mL round-bottom flask. Stirring this mixture for 1 h produced a solution of the CuCl(OH)−TMEDA complex (a dark blue solution). To this solution was added ethyl ferulate (2.22 g, 10 mmol) dissolved in 20 mL of acetonitrile, and this mixture was stirred for 100 min. The reaction was stopped by adding 150 mL of 1 M HCl, producing a clear green solution. The acetonitrile was removed by evaporation at 40 °C under reduced pressure, and the products were recovered by ethyl acetate extraction (150 + 100 mL). The ethyl acetate solution was washed with saturated NH_4Cl (100 mL) solution and dried over anhydrous $MgSO_4$. Filtration through a sintered glass funnel and evaporation of the ethyl acetate solution produced the crude product mixture.

Method B [CuCl(OH)−TMEDA-Catalyzed Oxidation]. A molar equivalent of TMEDA (116 mg, 1.0 mmol) was added into anhydrous CuCl (100 mg, 1.0 mmol) in acetonitrile (150 mL) in a 500 mL round-bottom flask, and this mixture was stirred for 5 min, resulting in a deep blue solution. Into the solution was added ethyl ferulate (2.22 g, 10.0 mmol), and an oxygen-filled balloon was fitted onto the flask. After the mixture was stirred for 4.0 h, TLC showed that only small amounts of ethyl ferulate remained. Then 60 mL of 1 M HCl solution was added to stop the reaction, and the organic solvent was removed by evaporation under reduced pressure at 40 °C. The products were recovered by ethyl acetate (2 × 150 mL) extraction using a separatory funnel, and the organic phase was washed with acidic NH_4Cl solution (10 mL of 1 M HCl plus 100 mL of saturated NH_4Cl). The organic solution was dried over anhydrous $MgSO_4$, and the inorganic solid was removed by filtration through a sintered glass funnel. The crude products were obtained after evaporation of the ethyl acetate solution at 40 °C under reduced pressure.

When carried out analogously in dichloromethane, the oxidative coupling reaction can be completed in 3.5 h. However, the total yield of diferulates was lower because more trimers and higher oligomers were formed.

Flash Chromatography Purification. Eluting solvents were solvent A, hexane, and solvent B, ethyl acetate containing 5% (v/v) ethanol.

The crude products from the coupling reactions described above were loaded with 6−8 mL of dichloromethane onto a Biotage snap

normal-phase prepacked column (100 g of silica gel, 2.5 × 19 cm), and chromatography was performed on an Isolera purification system equipped with a UV detector and a sample collector.

The diferulate products were eluted with solvent mixtures programmed as follows: flow rate, 40 mL/min; isotropic elution, 1200 mL (70% solvent A + 30% solvent B) followed by gradient elution increasing the percentage of B to 50% at 2000 mL and held with that solvent mixture until the end at the 2800 mL elution volume. The trace profile was monitored by UV detection at 280 nm, and fractions were autocollected using "mediate slope mode" in 16 × 125 mm test tubes (maximal volume = 18 mL). The initial waste volume was 400 mL.

Characterization of Diferulate Products. The isolated and purified diferulate products were fully characterized by the usual series of NMR experiments (^{1}H, ^{13}C, HSQC, HMBC, COSY). NMR spectra were recorded on a Bruker Biospin (Billerica, MA, USA) AVANCE 500 (500 MHz) spectrometer fitted with a cryogenically cooled 5 mm TCI gradient probe with inverse geometry (proton coils closest to the sample). Bruker's Topspin 3.1 (Mac) software was used to process spectra. About 10−30 mg of diferulate in acetone-d_6 was used for NMR characterization, and the central solvent peaks, δ_H/δ_C 2.04/29.8, were used as internal references.

*8−5-Coupled Diferulates 2 and 3 (E)-Ethyl 5-(3-ethoxy-3-oxoprop-1-enyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-3-carboxylate, 8−5-Diferulate **2**.* Diferulate **2** (Figure 1) was one of the major dimeric products from the CuCl(OH)−TMEDA catalyzed aerobic oxidative coupling reaction of ethyl ferulate as described above. Flash chromatography of the crude oxidation products gave a fraction (eluting solvent volume from 740 to 850 mL) containing a mixture of diferulate **2** and diferulate **4**. Diferulate **2** was crystallized from ethyl acetate−hexane as white needles (0.48 g, 21.6%). It has the same NMR data as those previously published:[17](NMR database,[18] compound 2019) NMR (acetone-d_6) δ_H 1.27 (3H, t, J = 7.0 Hz, B-Me), 1.29 (3H, t, J = 7.0 Hz, A-Me), 3.83 (3H, s, ArOMe), 3.91 (3H, s, ArOMe), 4.18 (2H, q, J = 7.0 Hz, B−CH_2−), 4.26 (2H, m, A−CH_2−), 4.43 (1H, d, J = 8.0 Hz, A-8), 6.03 (1H, d, J = 8.0 Hz, A-7), 6.42 (1H, d, J = 16.0 Hz, B-8), 6.84 (1H, d, J = 8.0 Hz, A-5), 6.91 (1H, br d, J = 8.0 Hz, A-6), 7.09 (1H, br s, A-2), 7.28 (1H, br s, B-6), 7.34 (1H, br s, A-2), 7.62 (1H, d, J = 16.0 Hz, B-7); δ_C 14.46 (B-Me), 14.61 (A-Me), 56.00 (A7), 56.22 (OMe), 56.40 (OMe), 60.54 (A−CH_2), 62.18 (B−CH_2), 88.32 (A8), 110.65 (A2), 113.10 (B2), 115.76 (A5), 116.60 (B8), 118.90 (B6), 120.17 (A6), 127.34 (B5), 129.40 (B1), 132.00 (A1), 145.22 (B7), 145.78 (B3), 147.91 (A4), 148.50 (A3), 150.92 (B4), 167.26 (B9), 171.07 (A9).

The mother liquor after crystallization contained mainly 8−*O*−4-coupled diferulate **4** that could be isolated from the residual diferulate **2** following a tetrabutylammonium fluoride (TBAF) treatment as described below for the preparation of diferulate **4**. Although CuCl(OH)−TMEDA oxidative coupling of ethyl ferulate produced 8−5-diferulate **2** in a lower (10%) yield, it still could also be crystallized from a mixture fractionated by flash chromatography. If diferulate **2** is the primary required compound, peroxidase-catalyzed coupling remains perhaps the most straightforward and highest yielding method.[17]

*(E)-Ethyl 2-[5-((E)-3-ethoxy-3-oxoprop-1-enyl)-2-hydroxy-3-methoxyphenyl]-3-(4-hydroxy-3-methoxyphenyl)acrylate, 8−5-Diferulate (Noncyclic) **3**.* Diferulate **3** is not a coupling product from oxidation of ethyl ferulate, nor is its feruloyl polysaccharide analogue present in plant cell walls.[19] However, the free acid of **3** is often detected in alkaline hydrolysates of grass cell walls because alkaline hydrolysis of diferulate **2**, the only 8−5-coupled diferulate from radical coupling reactions, produces significant amounts of the acid of **3** in addition to the free acid of **2**. Diferulate **3** is therefore a valuable diferulate for cleanly preparing its acid analogue. In this study, diferulate **3** was obtained from TBAF treatment of diferulate **2** as described below for the isolation of diferulate **4**. Diferulate **3**: NMR (acetone-d_6) δ_H 1.20 (3H, t, J = 7.0 Hz, Me), 1.24 (3H, t, J = 7.0 Hz, Me), 3.44 (3H, s, B−OMe), 3.96 (3H, s, A−OMe), 4.13−4.19 (4H, m, A/B−CH_2−), 6.48 (1H, d, J = 16.0 Hz, B-8), 6.70 (1H, d, J = 1.8 Hz), 6.71 (1H, d, J = 7.8 Hz, A-5), 6.83 (1H, dd, J = 1.8, 7.8 Hz, A-6), 7.0 (1H, d, J = 1.8 Hz, B-6), 7.39 (1H, d, J = 1.8 Hz, B2), 7.57 (1H, d, J = 16.0 Hz, B-7), 7.77 (1H, s, A-7); δ_C 14.58 (Me), 14.60 (Me), 56.42 (A−OMe), 56.60 (B−OMe), 60.45 (B−CH_2), 61.03 (A−CH_2), 110.12 (B2), 113.16 (A2), 115.60 (A5), 116.22 (B8), 124.84 (A8), 125.68 (B6), 126.33 (A6), 126.50 (B5), 127.25 (B1), 127.52 (A1), 141.33 (A7), 145.23 (B7), 147.78 (A3), 147.97 (B4), 148.90 (A4), 149.03 (B3), 167.27 (B9), 167.73 (A9) (NMR database,[18] compound 2019).

*8−O−4-Coupled Diferulate 4 (Z)-Ethyl 2-[4-((E)-3-Ethoxy-3-oxoprop-1-enyl)-2-methoxyphenoxy]-3-(4-hydroxy-3-methoxyphenyl)acrylate, 8−O−4-Diferulate **4**.* Flash chromatography of CuCl(OH)−TMEDA oxidation products produced a fraction (380 mg) containing diferulates **2** and **4**, which was allowed to crystallize from ethyl acetate/cyclohexane to produce 220 mg of diferulate **2**. The mother liquor solution was evaporated, and the resulting residual oil was dissolved in 10 mL of acetonitrile and treated with 100 mg of TBAF for 30 min. After the addition of 5 mL of 1 M HCl solution, the mixture was evaporated at 40 °C under reduced pressure to remove the organic solvent. The residue was partitioned between ethyl acetate (15 mL) and 10 mL of 1 M HCl and the extracted ethyl acetate phase was washed with saturated NH_4Cl and dried over $MgSO_4$. Filtration through sintered glass removed the solids, and evaporation gave the product mixture as a yellow oil, which was purified by flash chromatography using a Biotage snap silica gel (10 g) prepacked column eluted with hexane/ethyl acetate (3:1, v/v) to produce 100 mg of pure diferulate **4** (eluting solvent volume from 120 to 200 mL) and 20 mg of diferulate **3** (eluting solvent volume from 360 to 560 mL). Diferulate **4**: NMR (acetone-d_6) δ_H 1.21 (3H, t, J = 7.0 Hz, Me), 1.26 (3H, t, J = 7.0 Hz, Me), 3.73 (3H, s, A−OMe), 3.99 (3H, s, B−OMe), 4.15−4.23 (4H, m, A/B−CH_2−), 6.45 (1H, d, J = 16.0 Hz, B-8), 6.78 (1H, d, J = 8.5 Hz, B-5), 6.81 (1H, d, J = 8.5 Hz, A-5), 7.12 (1H, dd, J = 8.5, 1.8 Hz, B-6), 7.23 (1H, dd, J = 8.5, 1.8 Hz, A-6), 7.38 (1H, s, A-7), 7.46 (1H, d, J = 1.8 Hz, B-2), 7.23 (1H, d, J = 1.8 Hz, A-2), 7.58 (1H, d, J = 16.0 Hz, B-7); δ_C 14.46 (Me), 14.58 (Me), 55.84 (A−OMe), 56.40 (B−OMe), 60.57 (B−CH_2), 61.70 (A−CH_2), 112.17 (B2), 113.65 (A2), 114.39 (B5), 115.94 (A5), 117.46 (B8), 122.91 (B6), 125.14 (A1), 126.08 (A6), 128.08 (A7), 130.08 (B1), 138.29 (A8), 144.79 (B7), 148.22 (A3), 148.76 (B4), 149.42 (A4), 150.16 (B3), 163.70 (A9), 167.63 (B9). These data were consistent with those published (NMR database,[18] compound 2041, ethyl/methyl ester).

*8−8-Coupled Diferulates 5 and 6 Diethyl 7-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-6-methoxy-1,2-dihydronaphthalene-2,3-dicarboxylate, 8−8-Diferulate (Cyclic) **5**.* Pure diferulate **5** (250 mg), eluted at solvent volume from 1360 to 1600 mL, was obtained by a flash chromatography fractionation of crude products from CuCl(OH)−TMEDA catalyzed aerobic oxidation of ethyl ferulate. Diferulate **5**: NMR (acetone-d_6) δ_H 1.10 (3H, t, J = 7.0 Hz, A−Me), 1.24 (3H, t, J = 7.0 Hz, B−Me), 3.75 (3H, s, A−OMe), 3.88 (3H, s, B−OMe), 3.93 (1H, d, J = 3.5 Hz, A-8), 3.97−4.05 (2H, m, A−CH_2−), 4.13−4.16 (2H, m, B−CH_2−), 4.51 (1H, d, J = 3.5 Hz, A-7), 6.42 (1H, dd, J = 8.0, 1.8 Hz, A-6), 6.64 (1H, s, B-5), 6.66 (1H, d, J = 8.0 Hz, A-5), 6.78 (1H, d, J = 1.8 Hz, A-2), 7.10 (1H, s, B-2), 7.64 (1H, s, B-7); δ_C 14.36 (Me), 14.56 (Me), 46.42 (A7), 48.24 (A8), 56.13 (A−OMe), 56.30 (B−OMe), 60.86 (B−CH_2), 61.18 (A−CH_2), 111.98 (A2), 113.08 (B2), 115.52 (A5), 116.70 (B5), 120.91 (A6), 123.59 (B8), 124.39 (B1), 132.11 (B6), 135.28 (A1), 137.97 (B7), 146.22 (A4), 147.50 (B3), 148.12 (A3), 149.43 (B4), 167.03 (B9), 172.72 (A9). These data were consistent with those published (NMR database,[18] compound 2035, methyl analogue).

*(2E,3E)-Diethyl 2,3-Bis(4-hydroxy-3-methoxybenzylidene)-succinate, 8−8-Diferulate (Noncyclic) **6**.* Diferulate **6** was isolated in a fraction (eluting solvent volume from 1000 to 1180 mL) from flash chromatography of oxidation products of ethyl ferulate with CuCl(OH)−TMEDA as oxidant. This crude fraction (215 mg) was further purified by secondary chromatography using a 25 g SNAP column, resulting in pure diferulate **6** (130 mg, 5.8% yield) as a pale yellow oil. Diferulate **6**: NMR (acetone-d_6) δ_H 1.10 (3H, t, J = 7.0 Hz, Me), 3.73 (3H, s, Ar−OMe), 4.11 (2H, q, J = 7.0 Hz, −CH_2−), 6.78 (1H, d, J = 8.2 Hz, A-5), 7.09 (1H, br d, J = 8.2 Hz, A-6), 7.25 (1H, br

s, A-2), 7.81 (1H, s, A-7); δ_C 14.48 (Me), 55.97 (OMe), 61.19 (−CH_2−), 113.26 (2), 115.92 (5), 125.56 (6), 125.60 (8), 127.80 (1), 142.42 (7), 148.14 (3), 149.30 (4), 167.53 (9). These data were consistent with those published.[16]

5−5-Coupled Diferulate 7 (2E,2′E)-Diethyl 3,3′-(6,6′-Dihydroxy-5,5′-dimethoxybiphenyl-3,3′-diyl)diacrylate, 5−5-Diferulate 7. Diferulate 7, one of the major dimeric products resulting from CuCl(OH)−TMEDA oxidation of ethyl ferulate, was isolated (220 mg, 10%) (eluting solvent volume from 1720 to 1800 mL) by flash chromatography. More diferulate 7 could be obtained by TLC separation of the other mixture fractions containing 7. Diferulate 7: NMR (acetone-d_6) δ_H 1.26 (3H, t, J = 7.0 Hz, Me), 3.96 (3H, s, Ar−OMe), 4.17 (2H, q, J = 7.0 Hz, −CH_2−), 6.44 (1H, d, J = 16.0 Hz, H-8), 7.21 (1H, d, J = 1.5 Hz, H-6), 7.53 (1H, d, J = 1.5 Hz, H-2), 7.64 (1H, d, J = 16.0 Hz, H-7); δ_C 14.60 (Me), 56.46 (OMe), 60.45 (CH_2), 109.76 (2), 116.20 (8), 125.52 (5), 126.20 (6), 126.50 (1), 1145.58 (7), 147.46 (4), 148.84 (3), 167.39 (9). These data were consistent with those in our NMR database,[18] compound 2057.

RESULTS AND DISCUSSION

It is well-known that Cu(II)−amine complexes can react with phenols generating radical species and that coupling of the resulting radicals produces dimers, trimers, oligomers, and even polymers with C−C or C−O bonds depending upon the Cu(II)−amine complex and the coupling conditions used.[20,21] Because Cu(II)−amine complexes are readily available and a variety of Cu(II)−amine complexes can be prepared by various combinations of copper salts and tertiary amines, they have been widely used to make diphenols, phenolic oligomers, and polyphenols.[22] The commercially available CuCl(OH)−TMEDA is a stable, free-flowing solid, soluble in chlorinated solvents, ethanol, diethyl ether, methanol, and THF. It was first introduced by Noji et al. in 1994 as a convenient catalyst for aerobic oxidative coupling of 2-naphthols to make binaphthols.[23] Most oxidative coupling reactions of phenols catalyzed by CuCl(OH)−TMEDA produced biaryl compounds; that is, such coupling reactions favor forming carbon−carbon bonds at the *para-* or *ortho*-positions to the phenol.[24]

To date, CuCl(OH)−TMEDA has not been used for the coupling of phenols with conjugated double bonds at the *para*-position, such as isoeugenol, hydroxycinnamates, or hydroxycinnamyl alcohols. Although the CuCl(OH)−TMEDA complex is commercially available and can be readily prepared, CuCl(OH)−TMEDA generated in situ in this study was equally effective and even more convenient to use. Thus, CuCl(OH)−TMEDA generated in situ was used as either an oxidant or a catalyst to effect oxidative coupling of ethyl ferulate, aimed at developing simple and convenient protocols to prepare diferulate isomers.

In method A, in which an molar equivalent CuCl(OH)−TMEDA was used as the sole oxidant, the coupling reaction carried out in acetonitrile went to completion in about 1.5 h, producing various dimers resulting from 8−5-, 8−8-, 5−5-, and 8−*O*−4-couplings, as indicated by GC-MS analysis of the crude products (Figure 2). However, in method B, in which such oxidative coupling was performed catalytically (10% CuCl-(OH)−TMEDA), using an oxygen atmosphere to regenerate the catalyst in situ, the total yields of all isolated diferulates were higher than those obtained in method A (Table 1). Thus, a total of five diferulates can be formed in a CuCl(OH)−TMEDA-catalyzed aerobic oxidative coupling of ferulate, all isolable in pure form, in yields from 4 to 21%, following flash chromatography. At this moment, it is not clear why these two methods produced different results, especially in the yields of 8−8-coupled products. It may be speculated that, at relatively

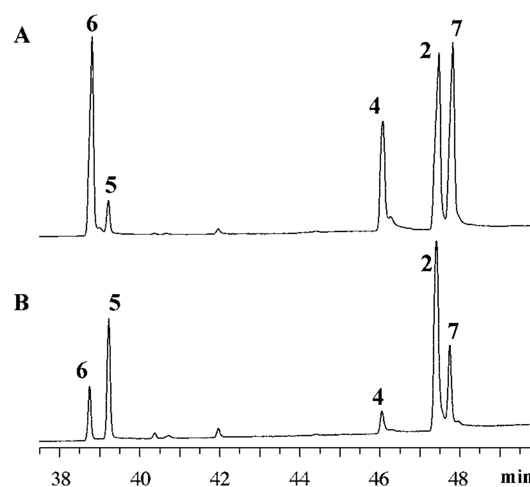

Figure 2. GC−total ion chromatography of TMS-derivatized dimeric products from (A) CuCl(OH)−TMEDA oxidation and (B) oxidative coupling of ethyl ferulate by CuCl(OH)−TMEDA catalyzed aerobic oxidation.

Table 1. Isolated Yields of Dimeric Products from Oxidation of Ethyl Ferulate 1

product description			
diferulate	no.	method A (noncatalytic)	method B (catalytic)
8−5-c	2	10.0	21.6
8−5-nc[a]	3	1.5	0.5
8−*O*−4-	4	4.0	4.3
8−8-c	5	−[b]	12.0
8−8-nc	6	5.6	4.5
5−5-	7	6.7	2.5
total		**27.8**	**43.4**

[a]Compound **3** was not produced directly from the coupling reaction of ethyl ferulate. [b]No attempt was made to isolate compound **5** due to its low yield via method A.

low concentrations of CuCl(OH)−TMEDA (method B), coupling of monomeric ethyl ferulate is favored, whereas at relative high concentrations of CuCl(OH)−TMEDA (method A) such preference (selectivity) is low so that trimers or oligomers are also produced significantly. It was observed that higher amounts of trimers or oligomers were indeed formed by method A than by method B.

As one of the major dimeric products in the CuCl(OH)−TMEDA oxidation system, the 5−5-coupled diferulate 7 was readily isolated in a modest yield by simple flash chromatography. More diferulate 7 could be obtained if the rest of the mixture containing 7 was fractionated again by chromatography. Diferulate 7 (or 5−5-diferulic acid) was the first dehydrodimer detected and isolated from plant cell walls.[25] The free acid of diferulate 7, often referred to simply as "diferulic acid", had been the sole diferulic acid found from plants until 1994, when other dehydrodimers including 8−5-, 8−*O*−4-, and 8−8-coupled dimers were identified thanks to syntheses and authentication of these dimers;[3] a further minor 4−*O*−5-dimer was later synthesized and authenticated from plants.[26] Although diferulate 7 was synthesized in a relatively easy way from vanillin,[3,27] several steps were required, and modest synthetic chemistry skills are still required to accomplish such a task. The protocol described here provides

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a simple alternative way to synthesize diferulate **7**. To the best of our knowledge, although it has been demonstrated that 5−5-diferulate **7** can be formed via coupling reactions initiated by various oxidation methods, this is the first time that diferulate **7** has been isolated in quantity, that is, on a preparative scale, directly from radical coupling of ferulate.

The 8−8-coupled diferulates (**5** and **6**) and their corresponding acids have been synthesized via multiple-step routes starting with ferulic acid;[3] the syntheses involved making the intermediate dilactone via oxidation of ferulic acid following a laborious workup. It has been reported that diferulate **5** can be obtained in 30% yield from radical coupling of ferulate by ferric chloride oxidation in aqueous acetone.[28] However, we were not able to obtain the claimed yield for diferulate **5** by following the exact procedure described in that paper. Instead, only 5% of diferulate **5** was isolated because huge amounts of unreacted starting materials remained. Diferulate **5** was detected in the coupling reaction products resulting from CuCl(OH)−TMEDA oxidation by method A, but isolation of pure **5** by flash chromatography was unsuccessful because of the extremely low yield produced. However, the CuCl(OH)−TMEDA catalyzed oxidative coupling of ethyl ferulate (method B) produced a much higher yield of diferulate **5**, and the pure **5** was isolated here in 12% yield by flash chromatography.

Diferulate **6** is another 8−8-coupled product, and the acid analogue has been frequently found in alkaline hydrolysates of grass cell walls and cereal bran since it was found and identified using the synthetic compound as a reference.[3] It was reported that diferulate **6** could be produced in 9% yield from oxidation of ethyl ferulate with alkaline potassium ferricyanide in a benzene−water two-phase system.[29] In this work, we were able to obtain pure diferulate **6** in 5.6% yield by a simple flash chromatographic separation of products from CuCl(OH)−TMEDA oxidation of ethyl ferulate (method A), in addition to 8−5-coupled diferulate **2**, 8−*O*−4-coupled diferulate **4**, and 5−5-coupled diferulate **7** produced from the same reaction. Similar yields of diferulate **6** can be obtained by executing the coupling reaction catalytically (method B), although a second purification by chromatography was needed to obtain pure **6**.

The synthesis of 8−*O*−4-coupled diferulate **4** was formerly the most challenging task, where up to nine steps were required to make diferulate **4** from vanillin and coniferaldehyde.[3] So far, this synthetic strategy remains the only way to produce **4** on a preparative scale. Although silver(I) oxide has been used frequently to make diferulate, there was only one report that diferulate **4** was obtained, in 17% yield from silver(I) oxide oxidation of methyl ferulate.[30] Unfortunately, we were not able to repeat the claimed results by following the published protocol with methyl or ethyl ferulate as substrate. In this study we demonstrated that diferulate **4**, produced by CuCl(OH)−TMEDA oxidation of ferulate **1**, can be isolated in two steps. Specifically, flash chromatography was first used to fractionate the oxidation products, producing a fraction containing diferulates **2** and **4** (Figure 1). This fraction mixture was allowed to crystallize in hexane−ethyl acetate, resulting in white needles of diferulate **2**. Then the residues after crystallization were treated with TBAF in acetonitrile to convert diferulate **2** into **3** while diferulate **4** remained intact. Diferulate **4** is readily separated from **3**; flash chromatographic separation led to isolation of the pure diferulate **4** in a yield of 4%, along with a small amount of diferulate **3**.

Although diferulate **3** is not a product resulting directly from coupling reactions of ferulate, the free acid derived from diferulate **3** is routinely found in alkaline hydrolysis products of grass plant cell walls and sometimes was mistakenly considered as a direct 8−5-coupling product from radical coupling of ferulate, a notion we have repeatedly tried to correct.[19] As its acid is a product resulting from cell wall saponification, however, it is still very useful to synthesize **3** for use as a standard compound to quantitate 8−5-coupled diferulate in plant cell walls. The free acid of **3** was one of the products produced by alkaline hydrolysis of diferulate **2**, whereas 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) treatment of diferulate **2** produced diferulate **3**.[3] In the current study, it was found that TBAF treatment of diferulate **2** in acetonitrile also resulted in diferulate **3** quantitatively, whereas diferulate **4** remained intact. Thus, TBAF can be used not only to aid in the isolation of diferulate **4** from a coeluting mixture containing **2** and **4** but also to obtain **3** from the readily available diferulate **2**,[17] from the peroxidase−H_2O_2 system, if quantities of diferulate **3** are needed.

In conclusion, it has been demonstrated that CuCl(OH)−TMEDA in acetonitrile is a robust biomimetic oxidation system for oxidative coupling of ferulate. Five diferulates, including 8−5-coupled **2**, 8−*O*−4-coupled **4**, 8−8-coupled **5**-**6**, and 5−5-coupled **7** diferulates, can all be formed and isolated in a pure form from CuCl(OH)−TMEDA-catalyzed aerobic oxidative coupling of ferulate in acetonitrile. To the best of our knowledge this is the first time that up to five pure diferulates have been isolated from one oxidative coupling reaction by flash chromatography; a simple TBAF treatment allows chromatographic separation of the 8−*O*−4-diferulate **4** from 8−5-diferulate **2**, with which it normally cochromatographs, and conveniently converts **2** to another valuable product, the open form of 8−5-diferulate **2**, that is, **3**, which is useful for cleanly producing its required carboxylic acid analogue. This synthetic approach allows diferulates needed for plant research to be prepared and isolated on a useful scale, without the need for skilled synthetic organic chemistry expertise.

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Notes

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