Simple Preparation of 8−5-Coupled Diferulate

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Diferulates are important products of ferulate-mediated cross-linking of plant cell walls. Coupling of ferulates by radical mechanisms produces a range of diferulates. One of the major isomers, 8−5-coupled diferulate, can be synthesized by single-electron oxidants, but the yields are relatively low and purification is nontrivial. Significant quantities of this dimer are required for various analytical and reactivity studies. A simpler preparation utilizing the biomimetic peroxidase−H_2O_2 system allows the production of gram quantities of pure diethyl 8−5-diferulate.

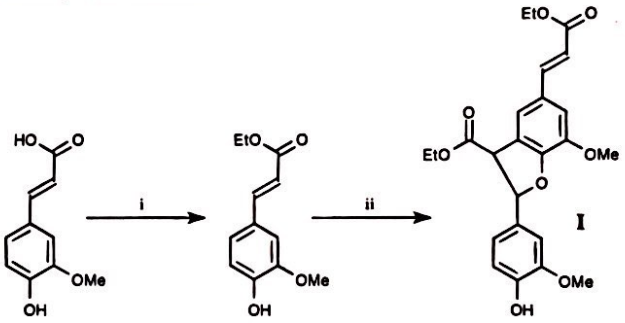
Keywords: *Ferulate; diferulate; ferulic acid; diferulic acid; peroxidase; radical coupling; cross-linking*

INTRODUCTION

Ferulates play important roles in plant growth and development. Polysaccharide−ferulate esters laid down in the plant cell wall undergo radical coupling reactions with other ferulates to produce ferulate dimers and effect polysaccharide−polysaccharide cross-linking (Ralph et al., 1994; Geissmann and Neukom, 1971; Fry, 1979) or with lignin units to effect lignin−polysaccharide cross-linking (Ralph et al., 1992, 1995). Ferulate dimers can also radically cross-couple with lignin units to effect even more substantial lignin−polysaccharide cross-linking (Quideau and Ralph, 1997; Ralph et al., 1997). Arising from radical processes, diferulates are produced in several regioisomeric forms, although this has only recently been recognized (Ralph et al., 1994). The 8−8-, 8−5-, and 8−O−4-coupled isomers, are now invariably found in higher concentrations than the 5−5-coupled isomer, which was previously the only diferulate noted (with the exception of photochemical [2 + 2] products).

Syntheses of all of the diferulates were reported with the initial discovery of the range of diferulates (Ralph et al., 1994). However, significant quantities are now required by cell wall researchers, and larger scale and simpler preparations are required. The 5−5-coupled dimer is relatively easily prepared, but the 8−O−4- and 8−8-coupled isomers remain in the domain of synthetic organic chemists. The 8−5-coupled product was prepared by a relatively simple method, utilizing silver oxide as the single-electron oxidant, but product separation was not trivial. It has been well established (Ralph et al., 1992; Teutonico et al., 1991; Chioccara et al., 1993; Wallace and Fry, 1995) that the primary ferulate dimer generated utilizing the biomimetic peroxidase−H_2O_2 system, or a range of single-electron oxidants, is the 8−5-coupled dimer, but a practical gram-scale preparation has not emerged. Here we report a simple preparation that produces pure 8−5-coupled diethyl diferulate **I** cheaply and in reasonable yields (Scheme 1).

Scheme 1. Biomimetic Synthesis of 8−5-Coupled Diethyl Diferulate I[a]



[a] (i) EtOH/HCl, yield ~100%. (ii) peroxidase, H_2O_2, pH 4, 10 min, yield ~50+%.

EXPERIMENTAL PROCEDURES

Ferulic acid and peroxidase (EC 1.11.1.7, type II, 150−200 units/mg, from horseradish) were obtained from Sigma; 30% H_2O_2 was from Mallinckrodt and acetyl chloride from Aldrich. Solvents were of AR grade. Petroleum ether was the 35−60 °C boiling fraction. All were used without further purification. Analytical TLC was performed with Alugram Sil-G/UV_{254} plates (Macherey-Nagel) with visualization by UV light. Silica gel flash chromatography was on a Biotage Flash 40M flash chromatography unit using a 70 g prepacked column (32−63 μm particles, 60 Å average pore size, 570 m^2/g surface area), utilizing an ISCO UA-6 UV−vis detector and an ISCO Foxy 200 fraction collector.

Ethyl Ferulate. Ethyl ferulate was prepared as described previously (Ralph et al., 1994) from ethanolic HCl, prepared conveniently according to Fieser's method (Fieser and Fieser, 1967). Thus, ferulic acid (10 g) was dissolved in absolute ethanol (100 mL) and acetyl chloride (5 mL) added. The solution was stirred gently overnight, and the volatiles were removed by rotary evaporation at 40 °C. Addition of further ethanol and evaporation several times removed the HCl. The product was then used directly for the diferulate step or allowed to crystallize from EtOAc/petroleum ether.

Dimerization. The ethyl ferulate (2 g) was dissolved, with heating (to ~60 °C), into pH 4.0 acetate buffer (2 L). After

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the solution cooled to ~40 °C, H_2O_2 (0.76 mL) was added and then peroxidase (10 mg in 2 mL of phosphate buffer). Within seconds, crude product began to precipitate out. After 10 min, the product was filtered through sintered glass yielding crude product (~2 g). Alternatively, the solution can be extracted directly with EtOAc.

Purification. TLC indicated that the required product was well separated from trace faster moving and more major slower moving uncharacterized contaminants. Flash chromatography using EtOAc–petroleum ether (30:70, ~30 mL/min) as eluant produced the product in fractions 37–50 (each 15 mL). Product **I** spontaneously crystallized on evaporation of the solvent (purified yield, 1.05 g, ~50%). Although not really required, recrystallization from acetone–petroleum ether produced white needle crystals. NMR data and melting point, 152.8–153.1 °C, were as detailed previously (Ralph et al., 1994).

DISCUSSION

Preparing phenylcoumarans of this type by purely synthetic methods without utilizing radical coupling reactions is tedious (Ede et al., 1987; Ralph et al., 1987; Brunow and Lundquist, 1984; Nakatsubo and Higuchi, 1979; Hise et al., 1985; Hassi et al., 1987). The silver(I) oxide method used previously (Ralph et al., 1994), and presumably other methods, produced the required product in ~30% yield, but it was contaminated by components from which separation was not trivial. The biomimetic peroxidase–H_2O_2 system, described under Experimental Procedures, produced the required 8–5-coupled product **I** quickly in reasonable yield; from the NMR spectra, the yield appeared to be very high, but polymeric components must have accounted for a substantial fraction. Importantly, those contaminants that were present had significantly different mobilities on silica, allowing simple separation. The yield of purified product was ~50%. Although we used a commercial flash chromatography system, the separation could be easily carried out by simpler flash systems (Taber, 1982; Still et al., 1978), the sintered glass funnel method (Leopold, 1982), or any other nonpressurized chromatography system. We loaded the full 2 g of product onto Biotage's silica 40M packed column and, within ~20 min, had the pure 8–5-coupled diferulate separated. Scaling up the reaction did not produce greater quantities of the required product; optimization studies at different scales were not pursued, however.

This simple method should allow laboratories to synthesize their own stocks of this valuable compound. Unfortunately, the only other dimer so readily prepared at this point is the 5–5-coupled dimer. Methods to produce high yields of 8–O–4- or 8–8-coupling, utilizing the single-electron systems described by Landucci (1995) for favoring these products in monolignol coupling reactions, are being explored but have so far been disappointing for diferulates.

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