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Short communication

Scaled-up production of poacic acid, a plant-derived antifungal agent

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ABSTRACT

Poacic acid, a decarboxylated product from 8–5-diferulic acid that is commonly found in monocot lignocellulosic hydrolysates, has been identified as a natural antifungal agent against economically significant fungi and oomycete plant pathogens. Starting from commercially available or monocot-derivable ferulic acid, a three-step synthetic procedure has been developed for the production of poacic acid needed for field testing in a controlled agricultural setting. First, ferulic acid was esterified to produce ethyl ferulate in 92% yield. Second, peroxidase-catalyzed free radical dehydrodimerization of ethyl ferulate produced crude diferulates, mainly 8–5-diferulate, in 91% yield. Finally, crystalline poacic acid was obtained in 25% yield *via* alkaline hydrolysis of the crude diferulates after purification by flash-column chromatography. This new procedure offers two key improvements relevant to large-scale production: 1) bubbling air through the reaction mixture in the second step to remove acetone greatly improves the recovery efficiency of the crude diferulates; and 2) telescoping minor impurities directly into the alkaline hydrolysis step eliminates the need for additional column purifications, thus reducing the overall cost of production and removing a major impediment to process scale-up.

1. Introduction

As the world population grows, fungicide-resistant pathogens pose a threat to agricultural sustainability and global food security (O'Maille, 2015; Piotrowski et al., 2015.) This threat has led to widespread use of fungicidal agrochemicals that may themselves put human health at risk and damage the environment (O'Maille, 2015). Copper-based fungicides, for example, show high efficacy in organic agriculture, but they now face restrictions due to copper accumulation in soils (Mackie et al., 2013; Piotrowski et al., 2015; Pose et al., 2009; Wightwick et al., 2010, 2013). Consequently, new (and safe) fungicidal agents are in high demand. Poacic acid **4** (Fig. 1.) derives from a major dehydrodiferulate (from here on termed simply diferulate), the 8–5-dimer **3** that is an analog of the native ferulate-arabinoxylan-derived esters present in grasses (monocots); poacic acid **4**, along with the acid analog of **3**, is released by saponification of grasses (Ralph et al., 1994). We recently synthesized this compound again and assessed its antifungal properties against several economically relevant fungi and oomycete plant pathogens, including *Sclerotinia sclerotiorum*, *Alternaria solani*, and *Phytophthora sojae* (Lu et al., 2012; Piotrowski et al., 2015). Its potency highlights the potential value of bioactive chemicals produced from lignocellulosic hydrolysates, which could help improve the overall economic balance of a biorefinery.

Poacic acid shows broad potential as a plant-derived fungicide, especially based on its efficacy against both fungi and oomycetes (Piotrowski et al., 2015). Moreover, in comparing its antifungal activity to that of other widely used agricultural fungicides, poacic acid offers some notable advantages. As mentioned, copper sulfate accumulates to toxic levels in soils (Eastman, 2016). whereas plant-derived poacic acid would be rapidly broken down in the soil and would therefore not accumulate (Chen et al., 2011; Piotrowski et al., 2015). As such, poacic acid could eventually find use as a fungicide in both sustainable and conventional farming, although additional field trials, application strategies, and more diverse pathogen tests must first be considered to evaluate its performance as an agricultural fungicide and its persistence in the environment.

In order to conduct field trials in a controlled agricultural setting, significant quantities of this compound are required. To date, there have been no methods developed for the large-scale syn-

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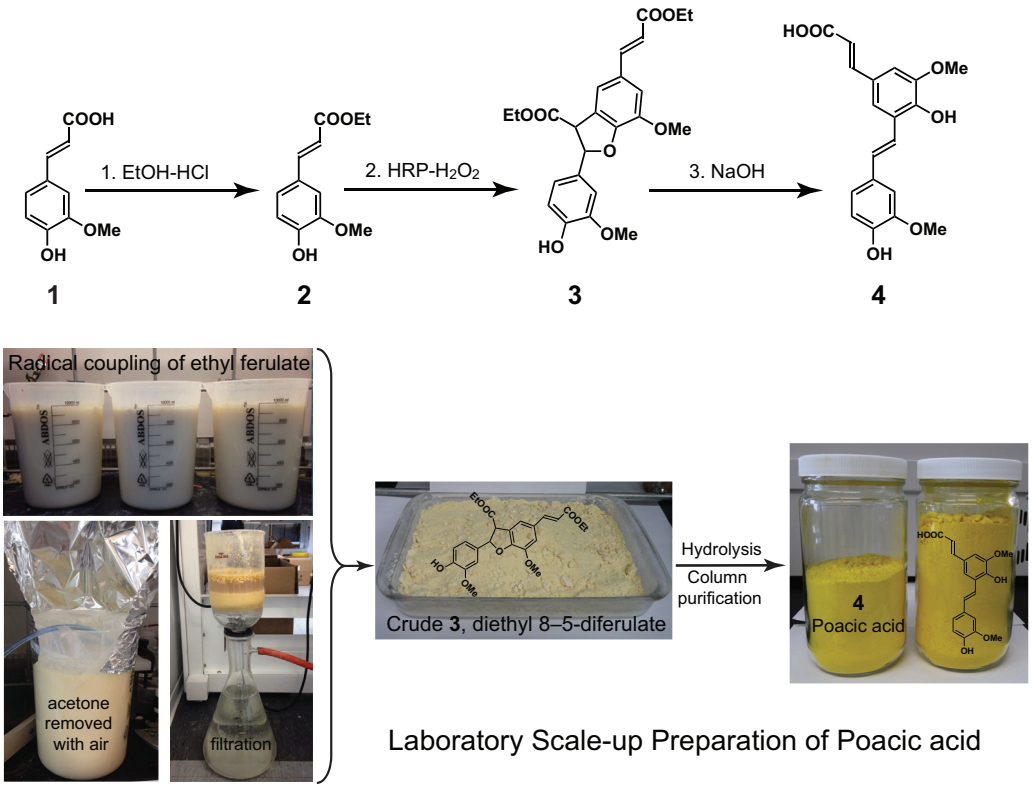

Fig. 1. Synthesis of Poacic acid **4**.

thesis of poacic acid, although several multi-step, milligram-scale, synthetic preparations have been reported (Bunzel et al., 2004; Lu et al., 2012; Neudorffer et al., 2003; Ralph et al., 1994). Recognizing a need for increased production to facilitate field trials, we developed an improved large-scale synthetic procedure that allows kilograms of produced crystalline ethyl ferulate to yield hundreds of grams of crude 8–5-diferulate that can be directly hydrolyzed to obtain poacic acid following flash chromatography with reasonable overall yields. This procedure could be adapted to make poacic acid on an industrial scale.

2. Materials and methods

2.1. Materials

trans-Ferulic acid **1**, a white to off-white crystalline powder, was purchased from Chem-Impex International Inc. (Wood Dale, IL, USA). All other chemicals and solvents used in this study were obtained from Aldrich (Milwaukee, WI, USA) and used as supplied.

Flash chromatography was performed on a Biotage® Isolera One (Biotage, Charlottesville, VA) instrument, using pre-packed (or re-packed) SNAP cartridges (340 g of silica gel). NMR spectra were acquired 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). Spectra were processed using Bruker's Topspin 3.5 (Mac) software. Standard Bruker implementations of one- and two-dimensional (gradient-selected COSY, HSQC and HMBC) NMR experiments were used for routine structural assignments of all synthesized compounds. The conditions used for all samples were 5–10 mg in 0.5 mL acetone-d_6, with the central solvent peak (δ_H/δ_C 2.04/29.80) used as an internal reference.

2.2. Synthesis of poacic acid

As shown in Fig. 1, poacic acid **4** was synthesized in three steps.

2.2.1. Synthesis of ethyl ferulate *2*

Ethyl ferulate **2** was made from *trans*-ferulic acid **1** (1.0 kg, 5.15 mol) and ethanolic HCl made by adding acetyl chloride (125 mL, 1.75 mol) to ethanol (2.5 L) as previously described (at smaller scale) (Ralph et al., 1994). Recrystallization from ethyl acetate/hexane afforded ethyl ferulate as light yellow crystalline needles in a yield of 92%. Compound **2**, NMR, δ_H: 7.58 (1H, d, J = 15.95 Hz, α), 7.33 (1H, d, J = 1.94 Hz, Ar-2), 7.13 (1H, dd, J = 8.18, 1.90 Hz, Ar-6), 6.86 (1H, d, J = 8.23 Hz, Ar-5), 6.38 (1H, d, J = 15.94 Hz, β), 4.17 (2H, q, J = 15.20, 7.57 Hz, $-\underline{CH_2}CH_3$), 3.91 (3H, s, OMe), 1.26 (3H, t, J = 7.16 Hz, $-CH_2\underline{CH_3}$); δ_C: 167.36 (γ), 149.95 (Ar-4), 148.67 (Ar-3), 145.45 (α), 127.39 (Ar-1), 123.86 (Ar-6), 115.98 (Ar-5), 115.89 (β), 111.13 (Ar-2), 60.43 ($\underline{CH_2}CH_3$), 56.24 (OMe), 14.61 ($CH_2\underline{CH3}$).

2.2.2. Synthesis of diethyl 8–5-diferulate *3*

Diethyl 8–5-diferulate **3** was produced *via* peroxidase-catalyzed radical dehydrodimerization of ethyl ferulate **2**, which was carried out in 10 L plastic beakers. Ethyl ferulate **2** (88 g, 0.40 mol) was dissolved in 1.8 L of acetone, and the solution was diluted to 7.0 L with purified (reverse-osmosis/deionized, RO/DI) H_2O. A solution containing 20.5 g H_2O_2-urea complex (1.1 eq) in 150 mL RO/DI H_2O was then added to the reaction mixture, followed by the addition of 40.0 mg of horseradish peroxidase (HRP) in 100 mL RO/DI H_2O. The reaction mixture was then diluted to 10.0 L with RO/DI H_2O and stirred at room temperature, with reaction monitoring by TLC (*n*-hexanes/EtOAc, 3:1 v/v). The solution turned into a yellow slush after 10 min, and the color remained until the end of the reaction. After approximately 35 min, TLC analysis showed almost full consumption of the starting ethyl ferulate. The reaction mixture was