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Synthetic Procedure of Pinoresinol

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DISCLAIMER

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ABSTRACT

A direct understanding of the degradation reaction pathways of lignin polymers in biomass is difficult due to the complexity of lignin's structure. To overcome the difficulty, simple lignin dimeric and trimeric model compounds which include typical lignin interunit linkages are useful to clarify reaction mechanisms. The following procedure describes the synthetic procedure of a β-β dimeric lignin model compound: pinoresinol. Lignin model compounds are useful for screening the effectiveness of catalysts and microoganisms. As well as determining the effect of a treatment on the lignin fraction, in particular the effect on the degree of depolymerization in the lignin polymer.

MATERIALS

Coniferyl alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #223735**
Step 1.1 (Can also be synthesized in a two-step reaction from ferulic acid)
Acetone **Fisher Scientific Catalog #A18P-4** Step 1.1
Iron (III) chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #236489**
Step 1.1
Ethyl acetate **Fisher Scientific Catalog #E145** Step 1.1 and 2.1
Sodium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S9888**
Step 1.1 [see Note 1]
Sodium sulfate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #239313**
Step 1.1
Hexane mixture of isomers **Merck MilliporeSigma (Sigma-Aldrich) Catalog #178918**
Step 2.1
Silca gel **Merck MilliporeSigma (Sigma-Aldrich) Catalog #60737** Step 2.1
Acetone-D6 **Cambridge Isotope Laboratories, Inc. Catalog #DLM-9-25ML**
Step 3.1
Chloroform-D **Cambridge Isotope Laboratories, Inc. Catalog #DLM-7-100**
Step 3.1

SAFETY WARNINGS

Almost all chemicals used for this procedure are hazardous. Read the Safety Data Sheet (SDS) for all chemicals and follow all applicable chemical handling and waste disposal procedures.

BEFORE START INSTRUCTIONS

All glassware is dried in an oven set to 105ºC then cooled in a desiccator prior to use.

Synthetic Procedure

1

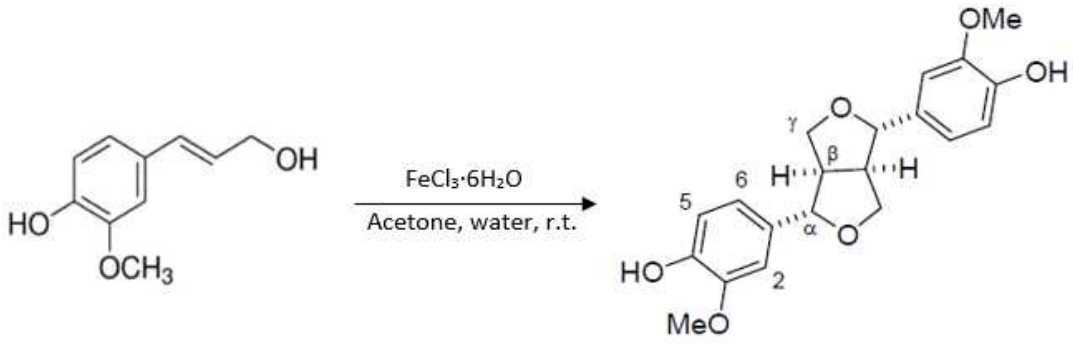

Figure 1. Reaction scheme for the synthesis of pinoresinol (β-β dimer).

1.1 Coniferyl alcohol (2.0782 g , 11.5 mmol) was dissolved in acetone (15 mL) then diluted with deionized (D.I.) water (70 mL). A solution of iron (III) chloride hexahydrate (3.4289 g , 12.7 mmol, 1.1 eq) in D.I. water (30 mL) was then added to the reaction mixture. The mixture was stirred at room temperature for 1 hour. After an hour, the reaction was extracted five times with ethyl acetate (70 mL x5). It was then washed with a saturated solution of brine (350 mL) [see Note 1], dried over sodium sulfate, and concentrated *in vacuo*.[1,2] The crude mixture was purified via flash chromatography to yield pinoresinol (0.4971 g, 24%) as well as β-O-4 (0.5507 g, 25%) and β-5 (0.261 g, 13%) dimer side products.

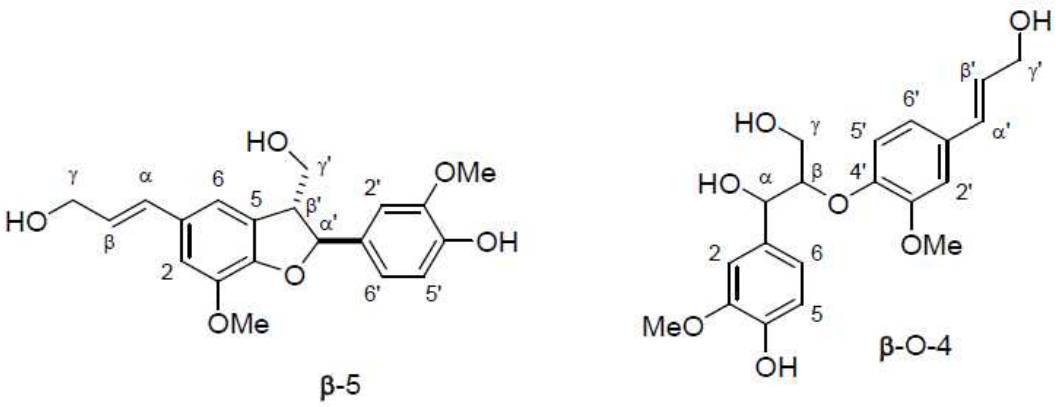

Figure 2. Chemical structures of β-5 and β-O-4 dimer side products.

Note

Note 1. Preparation of saturated brine solution: Fill a container partially with D.I. water. Add a spatula full of sodium chloride (NaCl) and stir until dissolved. Repeat until excess NaCl begins to settle onto the bottom of the container.

[1]

CITATION

C. S. Lancefield, N. J. Westwood (2015). The synthesis and analysis of advanced lignin model polymers. Green Chemistry.

LINK

10.1039/c5gc01334h

[2]

CITATION

Ciaran W. Lahive, Paul C. J. Kamer, C. S. Lancefield, Peter J. Deuss (2020). An introduction to model compounds of lignin linking motifs; synthesis and selection considerations for reactivity studies. ChemSusChem.

LINK

10.1002/cssc.202000989

Purification

2 Flash chromatography was performed using a Teledyne Isco Combiflash® NextGen 300+. Collected fractions were determined using a UV detector with wavelengths set at 254 and 280 nm. Samples were prepared by dissolving the crude material in the smallest amount of compatible solvent. Silica gel (mesh size 70-230) was then added to adsorb the material. Excess solvent was vacuum evaporated and the sample was loaded into a RediSep® R_f 25 g sample cartridge (catalog # 69-3873-240).

2.1 Pinoresinol was purified via flash chromatography. Column used was a RediSep® Silver 80 g silica gel flash column (catalog # 69-2203-380). Solvent system was hexane (Solvent A) and ethyl acetate (Solvent B). Pinoresinol was collected starting five minutes into the run using a ratio of 60% ethyl acetate: 40% hexane. The β-5 dimer side product was collected starting

twenty minutes into the run using a ratio of 85% ethyl acetate:15% hexane. The β-O-4 dimer side product was collected starting thirty-two minutes into the run using a ratio of 95% ethyl acetate:5% hexane.

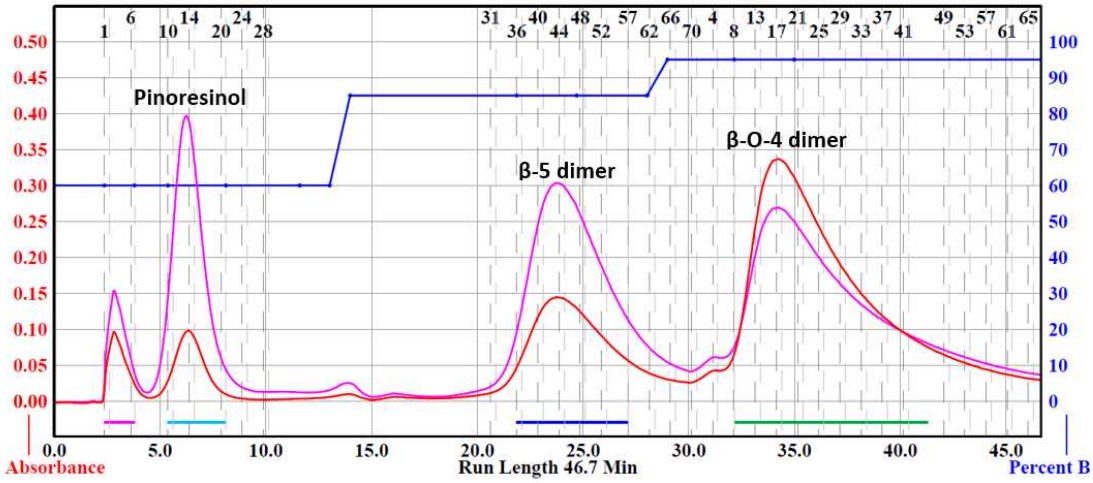

Figure 3. Run program from Combiflash® NextGen 300+ of pinoresinol separation as well as the β-5 and β-O-4 dimer side products.

NMR Spectroscopy

3 Nuclear magnetic resonance (NMR) spectra are acquired in suitable deuterated NMR solvent at 25°C on a Bruker AVANCE 400MHz spectrometer equipped with a 5 mm BBO probe. Chemical shifts (δ) are reported in ppm. ^{1}H-NMR spectra are recorded with a relaxation delay of 1.0 s and an acquisition time of 4.09 s. The acquisition parameters for ^{13}C-NMR include a 90˚ pulse width, a relaxation delay of 1.0 s, and an acquisition time of 1.36 s. Tetramethylsilane is used as a reference.

3.1

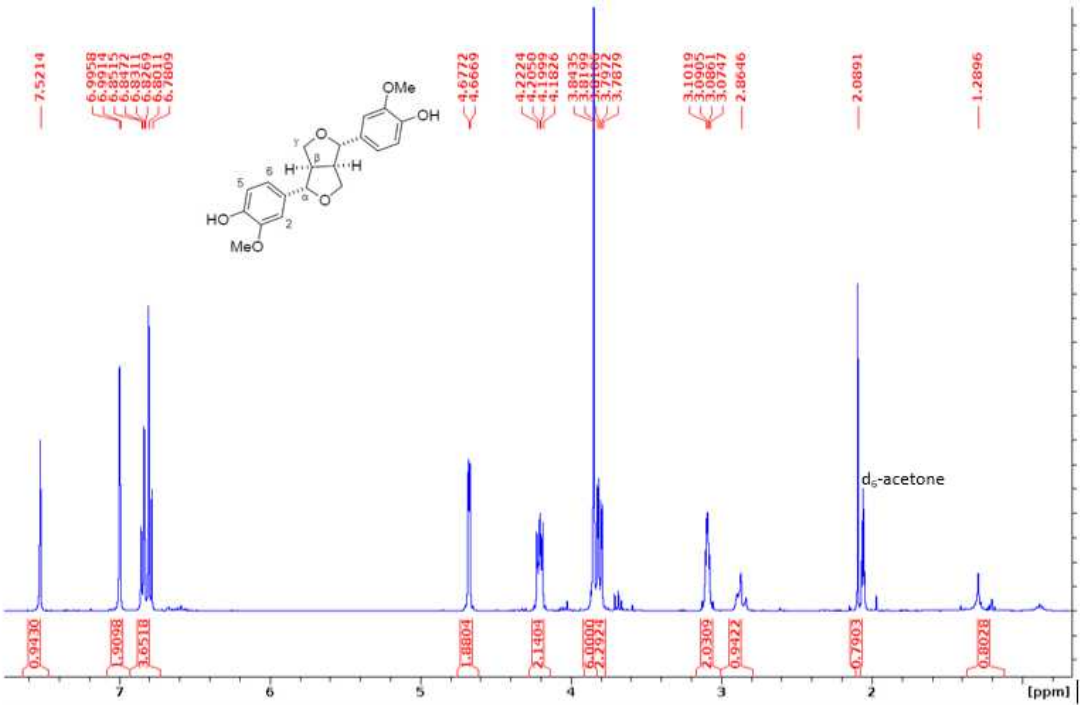

Figure 4. ^{1}HNMR spectrum of pinoresinol.

^{1}H NMR (400 MHz, d_6-acetone) δ 7.52 (s, 1H, ArOH), 6.99 (d, J=1.8 Hz, 2H, H2), 6.85-6.78 (m, J = 8.2, 1.7 Hz, 4H, H5/6), 4.67 (d, J = 4.1 Hz, 2H, Hα), 4.22 – 4.18 (m, J= 6.9, 2.0 Hz, 2H, Hγ), 3.91 (s, 6H, OMe), 3.84 – 3.78 (m, 2H, Hγ), 3.10 – 3.07 (m, J= 4.6, 1.8 Hz, 2H, Hβ).

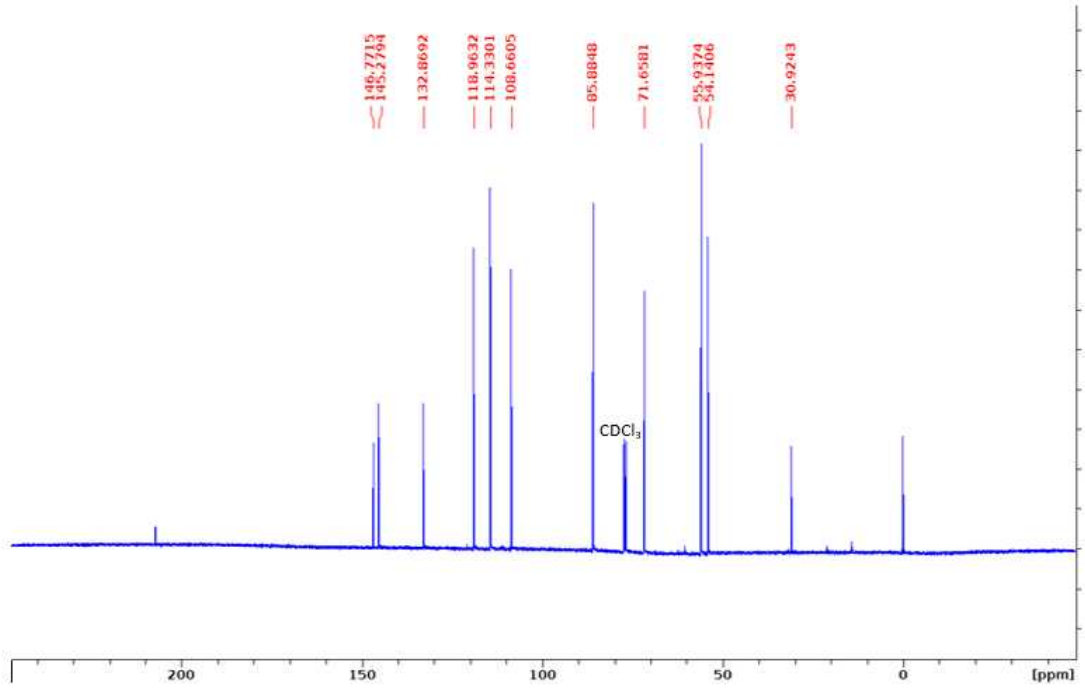

Figure 5. ^{13}C NMR spectrum of pinoresinol.

^{13}C NMR (100 MHz, $CDCl_3$): 146.77 (3), 145.28 (4), 132.87 (1), 118.96 (6), 114.33 (5), 108.66 (2), 85.88 (α), 71.66 (γ), 55.94 (OMe), 54.14 (β).

β-5 ^{1}H NMR (400 MHz, d_6-acetone): δ 7.67 (s, 1H, ArOH), 6.94-6.85 (m, 5H, aromatic region), 6.40 (d, J=1.6 Hz, 1H, Hα), 6.18 (dt, J=9.2, 6.6 Hz, 1H, Hβ), 5.10 (d, J=9.3 Hz, 1H, Hα'), 4.31 (d, J=5.9 Hz, 2H, Hγ), 3.86 (s, 3H, OMe), 3.85 (s, 3H, OMe), 3.84 (m, 2H, Hγ'), 3.46 (m, 1H, Hβ').

β-O-4 ^{1}H NMR (400 MHz, d_6-acetone): δ 7.42 (s, 1H, ArOH), 6.91-6.87 (m, 6H, aromatic region), 6.56-6.50 (m, 1H, Hα'), 6.34-6.31 (m, 1H, Hβ'), 4.91-4.87 (m, 1H, Hα), 4.53 (s, 2H, Hγ'), 4.22 (ddd, J=5.6, 3.6, 1.4 Hz, 0.5H, OHγ), 4.08 (dt, J=7.1 Hz, 0.5H, OHγ), 3.89-3.85 (m, 2H, Hγ), 3.82 (s, 3H OMe), 3.81 (s, 3H, OMe), 3.57-3.44 (m, 0.5H, OHα), 2.92 (dd, J=7.1, 5.6 Hz, 0.5H, OHγ'), 2.77 (dd, J=8.4, 5.6 Hz, 0.5H, OHγ').

[3]

CITATION

S. A. Ralph, L. L. Landucci, J. Ralph (2009). NMR Database of Lignin and Cell Wall Model Compounds.

LINK

https://www.glbrc.org/databases_and_software/nmrdatabase/NMR_DataBase_2009_Complete.pdf