Green Chemistry

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Green, Enzymatic Syntheses of Divanillin and Diapocynin for the Organic, Biochemistry, or Advanced General Chemistry Laboratory

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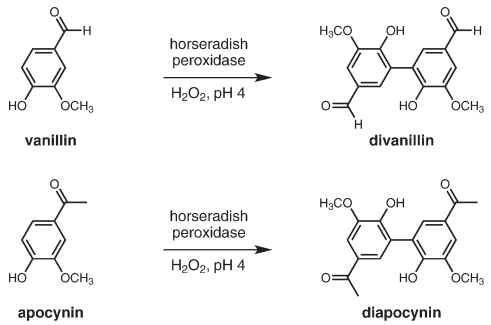
Vanillin and apocynin are versatile natural products and have been featured in various laboratory experiments for general or organic laboratory courses in this *Journal (1−5)*. However, few of these procedures would be considered green *(6−8)*. Here we describe a green, enzymatic preparation of the antioxidants divanillin and diapocynin that avoids the use of toxic reagents or inorganic salts (Scheme 1). Divanillin enhances the flavor of vanillin and can be formed by peroxidases during the curing process of vanilla beans *(9)*. Diapocynin may have an anti-inflammatory role, as it is a potent superoxide scavenger and is generated from apocynin by stimulated human polymorphonuclear neutrophils *(5, 10)*. The horseradish peroxidase-catalyzed *(11)* dimerization of vanillin or apocynin could be readily incorporated into an advanced general chemistry, organic, or biochemistry laboratory course.

Scheme 1. Oxidative Dimerization of Vanillin and Apocynin by Horseradish Peroxidase



Experiment Objectives

If this experiment is used in advanced general chemistry, students should

- perform a green, enzymatic synthesis of divanillin or diapocynin
- provide a balanced equation for the transformation
- isolate the product by filtration
- analyze the product by solubility, melting point determination, or ^{1}H NMR spectroscopy
- compare the environmental impact of this experiment with an alternative procedure

If this experiment is used in organic chemistry, students could additionally

- propose a mechanism for dimerization
- characterize the product by ^{1}H, ^{13}C, and two-dimensional NMR spectroscopy
- communicate their results in *Organic Letters* format

If this experiment is used in biochemistry, students could alternatively

- study the structure, mechanism, and reaction scope of horseradish peroxidase

Experimental Overview

Add vanillin (1.0 g, 6.6 mmol) or apocynin (1.1 g, 6.6 mmol) to 100 mL of deionized water in a 125 mL Erlenmeyer flask open to the air. Heat the mixture to dissolve the solids. Remove the solution from the heat source and add acetic acid (0.010 M in water, 2.2 mL, 0.022 mmol) to lower the pH to 4. At 40 °C or below, add horseradish peroxidase (Type I, 9.0 mg, 1000 units of activity) and then hydrogen peroxide (3% in water, 7.5 mL, 6.6 mmol) to the solution while stirring. Allow the reaction to stir for 5 min and then filter the tan precipitate using a Buchner funnel, rinsing the solids with deionized water. Allow the product to dry in air or a 50 °C drying oven, expecting a yield of 80−95%. Determine the melting point or record a ^{1}H NMR spectrum in DMSO-d_6 and compare the results to literature values. Advanced students may also obtain ^{13}C and ^{1}H−^{13}C HMQC spectra to aid in the assignment of signals to the structure. Mass spectrometry may also be used *(5)* if appropriate instrumentation is available (e.g., LC−MS or MALDI; the products are not well suited for GC−MS).

Hazards

Apocynin and dimethyl sulfoxide-d_6 are irritants. Dimethyl sulfoxide can carry other chemicals through the skin and should therefore be used with caution. Hydrogen peroxide may be harmful if swallowed and can cause eye irritation. Acetic acid is corrosive and is harmful if inhaled.

Discussion and Conclusions

For advanced general chemistry or nonmajor students, this experiment provides a good opportunity to get hands-on experience with green chemistry and an enzymatic reaction. Having students formulate a balanced equation helps to emphasize the point that water is the only byproduct formed and leads easily to discussions about environmentally friendly reaction design.

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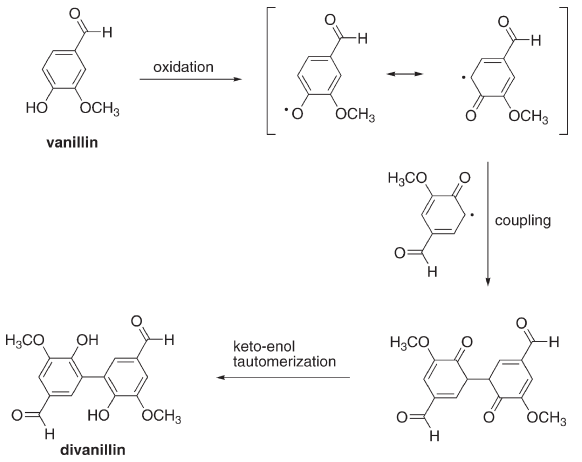
Scheme 2. Phenoxy Radical Coupling and Keto−Enol Tautomerization in the Synthesis of Divanillin



Melting point analysis is easy to perform, and even if the students have not learned about NMR spectroscopy, they can readily compare their spectrum to those of starting material and product standards.

For students in organic or biochemistry courses, the instructor can emphasize the bioorganic chemistry of oxidative phenolic coupling and keto−enol tautomerism (Scheme 2) or the bioinorganic chemistry of heme iron enzymes *(11−14)*. This experiment could also be an entry point for discussions about natural product biosynthesis. The use of HMQC NMR spectroscopy is particularly interesting because it helps to distinguish coincident aromatic hydrogen signals in the ^{1}H spectrum, and ^{13}C spectroscopy reveals a diagnostic downfield shift for the carbon at the new biaryl bond.

This experiment is a green alternative to the syntheses of divanillin *(15, 16)* and diapocynin *(5)* with stoichiometric inorganic oxidants. It is based on previous enzymatic, oxidative phenolic coupling procedures with vanillin and related compounds *(9, 16−19)*. Very little energy input is required, and filtration is among the easiest possible methods for product isolation. The product is sufficiently pure to distinguish it from the vanillin or apocynin starting material by NMR spectroscopy or melting point analysis. Students who perform this experiment appreciate that it directly produces compounds with valuable flavoring and medicinal properties from inexpensive starting materials, with water as the only byproduct. Few reactions are so mechanistically interesting, simple to perform, and green.

Acknowledgment

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Supporting Information Available

Instructions for students; notes for instructors; a one-page handout on green chemistry; NMR spectra for vanillin, divanillin, apocynin, and diapocynin in DMSO-d_6. This material is available via the Internet at http://pubs.acs.org.

