Green Synthesis of a Fluorescent Natural Product
A lab developed by Douglas M. Young, Jacob J. C. Welker, and Kenneth M. Doxsee, Department of Chemistry, University of Oregon

Lab Objectives:
1) To synthesize 4-methylumbelliferone by a Pechmann condensation.
2) To characterize the product by 1H NMR, IR, melting point and qualitatively by fluorescence.
3) To consider the effects that an easily recoverable, recyclable catalyst has on the atom economy and the e-factor of the reaction.
4) To consider the effects that a solventless reaction has on atom economy and e-factor of the reaction.
5) To test the effect of pH on fluorescence.

![Diagram of reagents and product]

Background:
Coumarins are natural products occurring in a variety of plants, including those used as traditional herbal medicines dating to as early as 1000 C.E. (1). The physical and chemical properties of the coumarins have been exploited in a wealth of practical applications, ranging from cosmetics, sunscreens, flavorings, and laser dyes (2) to pharmaceuticals (1, 2), including well-known anticoagulants ("blood thinners") (3). Umbelliferone, or 7-hydroxycoumarin, and a number of its methyl derivatives are found in plants of the family Umbelliferae, including carrots, parsley, cumin, and celery. Umbelliferones have recently found applications in fluorometric enzyme assays (4, 5) and as blood-brain barrier probes (6), anti-inflammatory agents (7), dyes (8), and fluorescent pH indicators (6, 9).

Hazards. Standard laboratory safety procedures should be followed. Resorcinol may be harmful if it is absorbed through open wounds or ingested, though symptoms appear to arise only upon chronic exposure. Ethyl acetoacetate, Dowex 50WX4 (acid form), and the product, 4-methylumbelliferone, can act as irritants; use of gloves and eye protection is advised. Ethanol is flammable, and hydrochloric acid and sodium carbonate solutions are corrosive and should be handled with care.

Experimental Procedure
1. In 50 mL Erlenmeyer flask combine ethyl acetoacetate (1.0 mL, 7.8 mmol), resorcinol (800 mg, 7.3 mmol) and Dowex 50WX4 beads (1.0 g).
2. In two additional 50 mL Erlenmeyer flask add 20 mL water to one and 15 mL ethanol (95%) to the other. (Place a pipette or spatula in flasks to prevent bumping.)
3. Place all three flasks on a hot plate and set to lowest setting.
4. Occasionally swirl and stir with a glass stir rod until the reagents cease bubbling and a grey solid begins to form (20-30 min).
5. When the reaction is complete add 2 mL hot ethanol (95%) to dissolve the solid.
6. Using a Pasteur pipette, pipette the hot ethanol solution from the Dowex beads into a clean Erlenmeyer flask.
7. If necessary, repeat steps 5 and 6 up to two more times, consolidating all three ethanol washes (keep warm).
8. While heating, carefully add hot water to the ethanol solution until the solution starts to become cloudy.
9. Allow to slowly cool to room temperature.
10. Collect the white to off-white precipitate by vacuum filtration and wash with water.
11. Allow to dry before taking the melting point and characterizing by IR and 1H NMR spectroscopy.
12. To observe fluorescence, dissolve a small amount (20 mg) of product in 2-3 mL of ethanol (95%) in three test tubes or scintillation vials. Alter the pH of two of the samples by adding 2 mL of a 10% HCl solution in one and 2 mL of a 10% Na<sub>2</sub>CO<sub>3</sub> solution to the other. Place samples under a UV lamp.

Post-lab Questions
1) Propose a mechanism for the acid-catalyzed Pechmann condensation of resorcinol and ethyl acetoacetate. Identify the transesterification, electrophilic aromatic substitution and dehydration steps.
2) Would you expect 1,3,5-trihydroxybenzene (phloroglucinol) to react more quickly or more slowly than resorcinol? Why or why not?
3) Consider and discuss the greenness of this reaction under:
   a. The original Pechmann reaction conditions in concentrated sulfuric acid
   b. This lab’s reaction conditions
4) Propose a transformation of 4-methylumbelliferone. Include reaction conditions and expected product(s).
5) Discuss and assign the peaks (chemical shift, splitting pattern, etc) in the 1H NMR and IR spectra of your product and the effect of pH on the product fluorescence.
6) Draw 4-methylumbelliferone in its protonated and deprotonated state. Be sure to include all significant resonance structures.

References: