Green Synthesis of a Fluorescent Natural Product

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Coumarins (Figure 1) 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)*.

The Pechmann condensation produces coumarins via the acid-catalyzed reaction of a phenol with a β-keto ester *(10–12)*. Whereas high temperatures and extreme reagents such as H_2SO_4 and $POCl_3$ were originally used to effect the transformation *(11, 13)*, the reaction has been improved upon by the development of protocols involving recyclable, solid acid catalysts *(14–18)*. In this *Journal*, Holden and Crouch reported a Pechmann reaction suitable for the undergraduate laboratory curriculum *(19)*. Utilizing a safe, easy-to-handle alternative to the above-mentioned reagents, the acidic ion exchange resin Amberlyst-15, this procedure represents an example of the successful modification of a chemical process through green chemistry.

Presenting a group of chemistry teachers with the opportunity to perform this preparation in a workshop convened in Mérida, Yucatán *(20)*, however, we were surprised when *none* of the workshop participants chose to do so. Discussions with the participants revealed a simple but unexpected reason. In the Holden and Crouch procedure, the reaction is effected in refluxing toluene, with a rotary evaporator used to remove the solvent once the reaction is complete. None of the participants had rotary evaporators available for teaching purposes at their home institutions, and none felt they could afford them, so they saw no reason to gain experience with an experiment that they simply could not use.

With this inspiration, we soon found that we could perform the reaction *without any solvent*, thereby avoiding both the requirement of a rotary evaporator and the use of the volatile solvent toluene that, although safer than benzene, still presents a number of safety, handling, and disposal issues *(21)*. Thus, 4-methylumbelliferone, **2**, can be rapidly synthesized by simply heating resorcinol, **3**, and ethyl acetoacetate, **4**, in the presence of a strong-acid ion exchange resin (Dowex 50WX4) (Scheme 1), enhancing the green chemical relevance of the experiment and making it more accessible even to those teachers with limited budgets.

The target umbelliferone **2** is nontoxic and easy-to-prepare and isolate, making it a particularly attractive target molecule for an undergraduate laboratory course. Also, **2** displays pH-dependent fluorescence that is easily observed with a standard UV lamp, providing students with a colorful deviation from the standard off-white powders they are accustomed to making *(9)*. Finally, the experiment can easily be adapted to open-ended student exploration of alternative substrates and catalysts.

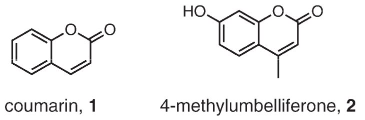
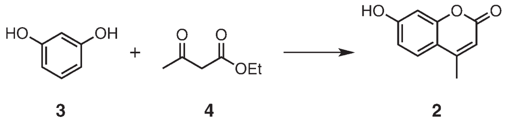


Figure 1. Structures of representative coumarins.

Scheme 1. The Pechmann Condensation



Experimental Details

A 50 mL Erlenmeyer flask is charged with ethyl acetoacetate (1.0 mL, 1.02 g, 7.8 mmol), resorcinol (800 mg, 7.3 mmol), and Dowex 50WX4 beads (1.0 g). The flask is placed on a hot plate set to the lowest setting. The reaction proceeds at a reasonable rate at temperatures as low as 80 °C, and commencement of the reaction is indicated by gentle bubbling. The mixture is occasionally swirled or stirred with a glass rod until bubbling ceases and the mixture solidifies to a tan solid (typically 20–30 min). A small volume, 2–3 mL, of hot ethanol (95%) is added to dissolve the solid and then a Pasteur pipet is used to transfer the hot solution from the Dowex beads to a clean Erlenmeyer flask. If desired, the beads may be rinsed with an additional portion of hot 95% ethanol. While heating, hot water is added until the solution becomes slightly cloudy and then the flask is removed from the hot plate and the mixture is allowed to cool slowly to room temperature. The white to off-white crystalline precipitate is isolated by vacuum filtration and is washed with water. The product is allowed to dry before determining the melting point and carrying out spectral analysis. Typical student yields range from 50 to 65% with a melting point of 179–181 °C (lit. 181–183 °C) *(14)*. NMR spectral analysis of the mother liquor usually reveals only the desired product, though occasionally unreacted resorcinol remains. Recrystallization to afford a second crop of crystals generally affords an additional ca. 10% yield. We have achieved comparable product yields and purity using 0.100 g of resorcinol (0.91 mmol), 0.125 mL ethyl acetoacetate, and 0.125 g Dowex 50WX4.

To observe fluorescence, a small quantity (ca. 20 mg) of the product is dissolved in 2–3 mL of 95% ethanol in each of three test tubes or scintillation vials. The pH of two of the samples is

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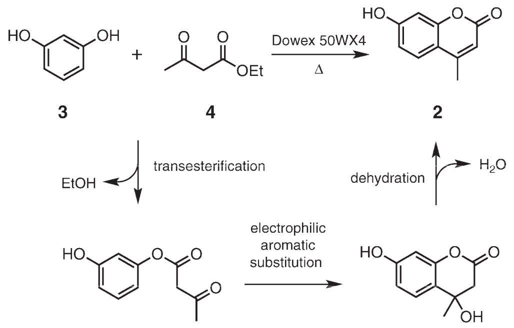
Scheme 2. The Elementary Reactions That Comprise the Pechmann Condensation



adjusted by adding 2 mL of 10% HCl to one and 2 mL of a 10% Na_2CO_3 solution to the other. The colors of the three solutions are compared under ambient light and under a long-wave UV lamp.

Hazards

Standard laboratory safety procedures should be followed. Resorcinol may be harmful if absorbed through open wounds or ingested, though symptoms appear to arise only upon chronic exposure *(22)*. 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.

Discussion

Although perhaps not as well-known as other "named" reactions *(23)*, the Pechmann condensation represents a rich source of teaching material, proceeding via a cascade of common reaction types: transesterification, intramolecular electrophilic aromatic substitution, alcohol dehydration (Scheme 2). Each of these steps is acid-catalyzed, providing students with the opportunity to explore mechanisms that illustrate the diversity of reaction types that can be effected through the mediation of a simple proton (see the supporting information).

The synthesis of 4-methylumbelliferone using Dowex 50WX4 under solvent-free conditions provides the framework for a discussion of a number of green chemistry principles *(24)*. The reaction is quite atom economical (principle 2), with only 1 equiv of ethanol and 1 equiv of water generated as byproducts *(25)*. The modified reaction avoids use of hazardous reagents such as sulfuric acid or phosphoryl chloride (principle 3). Avoidance of an organic solvent eliminates the associated hazards as well as reduces waste, and the purification method, a simple recrystallization from aqueous ethanol, avoids chromatography, often a major source of waste generation, in the form of the spent solid support and the elution solvent(s) (principle 5). Although the reaction requires elevated temperature, it is effected at a lower temperature than that reported previously, thereby reducing energy consumption and costs (principle 6). Waste is minimized by utilizing a mild, reusable acid catalyst that is easy to work with and recover, rather than with a stoichiometric auxiliary reagent (principle 9). We have reused the Dowex resin four times with an overall decline in yield of only 5%. The conversion from a liquid reaction mixture to a solid product effectively signals when the reaction is complete, representing a simple demonstration of the meaning of "real-time analysis" (principle 11). Use of an acid catalyst in the form of easy-to-handle nonvolatile beads makes handling this acid catalyst safer (principle 12). Guided discussion with students is encouraged, with such discussions often generating observations regarding the relevance of the experiment to additional principles of green chemistry.

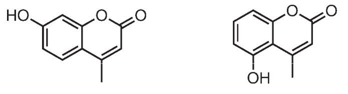

2 **5**

Figure 2. Possible isomeric products of the Pechmann condensation.


5-hydroxy-4-methylumbelliferone, **6** 4,7-demethyl-5-hydroxycoumarin, **7**

Figure 3. Additional umbelliferone compounds that could be synthesized.

This experiment also provides students with the opportunity to hone their interpretative skills by analyzing IR and ^{1}H NMR spectra (copies of which are available in the supporting information). Noting that the intramolecular electrophilic aromatic substitution reaction could conceivably occur at the ortho position between the two aromatic substituents, a reaction that would lead to the formation of the isomeric coumarin, **5** (Figure 2), students can speculate as to which isomer will form and then confirm or reject their hypothesis using the ^{1}H NMR spectral evidence. Moreover, the ^{1}H NMR spectrum of **2** is particularly instructional because it provides students with an example of long-range proton–proton coupling; both four-bond allylic coupling and five-bond aromatic para-coupling are observable.

The fluorescence in solution of coumarin **2**, and the pH dependence of this fluorescence *(9)*, easily observable using a simple handheld long-wave UV lamp, adds to the attractiveness and potential utility of this experiment. Although we have not done so, these observations could be quantified through examination of **2** by UV–visible and fluorescence spectroscopy. Depending on student level and interest, the origin of the fluorescence and its pH dependence can be explored through a literature survey, through which students can gain experience with a variety of more advanced concepts, including the alteration of pK_a in photoexcited states *(26)*.

Other phenols also react with ethyl acetoacetate to form coumarins under the reported reaction conditions, suggesting natural directions in which this procedure could be explored in a more open-ended way. For example, we found that analogous reactions of phloroglucinol and orcinol afford 5-hydroxy-4-methylumbelliferone, **6**, and 4,7-dimethyl-5-hydroxycoumarin, **7**, respectively (Figure 3). Because the specific coumarins obtained in such an open-ended study may present greater hazards than the innocuous coumarin **2** arising from resorcinol, critical evaluation of known physical and biological properties should be a required element of each student's prelab planning process.

Conclusion

The synthesis and analysis of 4-methylumbelliferone is suitable for an undergraduate organic chemistry lab course. Performing the Pechmann condensation introduces students to several types of organic reactions, transesterification, electrophilic aromatic substitution, alcohol dehydration, and provides students with an opportunity to interpret IR and ^{1}H NMR spectra to help elucidate the structure of their product. Performed with a recyclable, solid catalyst and under solvent-free conditions, the experiment illustrates many of the principles of green chemistry, serving as an excellent platform for the introduction and discussion of these principles. Finally, the fact that financial considerations, the high cost of rotary evaporators, led to development of a greener experiment illustrates the potential of green chemistry to yield not only safety and environmental benefits, but positive economic impacts as well.

Acknowledgment

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Supporting Information Available

Experimental details and tips, objectives, postlab questions, mechanisms, IR and ^{1}H NMR spectra for 4-methylumbelliferone. This material is available via the Internet at http://pubs.acs.org.