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Abstract

A laboratory experiment for natural aldehyde extraction and successive synthesis of new fluorophores is proposed. Characterization of the extracted aldehydes by different techniques and photophysical study of the luminescent products allow the design of new fluorophores for possible down-shifting, microscopy, and electronics applications. The fluorophores are generated by an easy one-pot cyclization reaction in mild conditions without catalyst and with only water as byproduct, the obtained compounds are characterized by mass spectrometry, NMR, electronic absorption and emission spectroscopy.

1. Introduction

Green engineering, environmental engineering, ecotechnology and sustainable technology are all equivalent definitions for the design of products and processes, which could be more efficient from an environmental standpoint; green engineering includes four basic approaches: waste reduction, materials management, pollution prevention, and product enhancement.(Favre et al., 2008; Hall and Howe, 2010; Harris and Briscoe-Andrews, 2008) These principles directed the strategy of the Reaction Engineering experimental procedure reported here, based on the natural product extraction and related synthesis of fluorescent products, with an eco-compatible synthetic approach.

In general, the student laboratory experiences must be both practical and inexpensive to replicate. In addition, these experiments must ensure the safety of the undergraduate students and the location hosting the experience itself, using safe reagents, solvents and processes, while minimizing the production of byproducts and wastes. Although syntheses of dyes and fluorophores have been present in classroom experiments for long time, few experiments are specifically related to the synthesis and use of different fluorophores.(Goodrich et al., 2015; Hutt and Aron, 2014; Macey et al., 2018; Young et al., 2011) Moreover, to the best of our knowledge, none of them allow students to prepare new fluorescent molecules starting from reagents extracted from natural materials. Therefore, an experience like the one proposed here was the unique result of a new educational approach to different aspects of the skills of an undergraduate student, here combined together.

The objective of the experiment proposed in this article was to provide students with an experience regarding two of the major areas common to chemistry: (1) the extraction of natural compounds using advanced techniques, such as steam-distillation and liquid-phase extraction, which students have yet to encounter, and (2) synthesis with a one-pot synthetic green approach and the photophysical characterization of the fluorescent products, minimizing byproducts and reducing the environmental impact.

The first purpose of this experiment was to introduce undergraduate students in a chemistry laboratory to the extraction of a natural product. In one 4 h lab period, students used steam-distillation and liquid-phase extraction to isolate a single relevant natural aldehyde from an aromatic plant. The identity of the molecule was verified using thin-layer chromatography, infrared spectroscopy, NMR and mass spectrometry.(Bott and Wan, 2013)

The second purpose of the experiment was to employ the extracted natural compound to synthesize new fluorophores with a one-pot synthetic green approach and to perform the photophysical characterization of the products. Indeed, luminescence is a multidisciplinary subject that encompasses synthetic, analytical and physical chemistry, with a large field of applications in microscopy, electronics and functional materials, making it perfect for an advanced chemistry laboratory course.(Fukaminato, 2011; Patterson et al., 2013).

The use of available and well known spices made the experiment close to everyday life experience. Moreover, we reported a list of different edible aromatic plants or seeds, containing different aldehydes, which are easy to find and buy at low price. Plants have long been documented as a major source of an exceptionally wide range of organic compounds. Extracts obtained from seeds, leaves, flowers and fruits of aromatic and medicinal plants present a large number of biological products, which make them interesting for food, cosmetic, pharmaceutical and chemical industries.(Breitmaier, 2006; Logan and Rumbaugh, 2012; McLain et al., 2015; Sequin, 2005)

In the second part of this experiment, the strong luminescence of the products was easily observable with a common UV lamp and had a strong emotional and cognitive impact on students.

The teaching and learning purposes of the laboratory experience were:
(i) to introduce chemical engineering students to a different synthetic strategy, based on green engineering, eco-technology and sustainable technology; (ii) to prove to students how their education relate to the concrete practice in the 'real world'; (iii) to obtain new interesting fluorescent products from natural products, with potential interest in different technological fields; (iv) to introduce students to cutting edge manipulative research in chemical engineering, so as to spark their curiosity. (v) to show the general and fundamental relation between the chemical structure and optical and electronic properties.
This Research Paper addresses the hypothesis that it is possible to prepare luminescent compounds of technological interest, starting from natural products, employing procedures inspired by the green engineering. Moreover, high yields, absence of catalysts and only water as a byproduct, no toxic and low-cost starting materials, ease of handling and preparation make this procedure and synthetic approach useful for a chemistry laboratory experience, and systematic screening of a large number of these compounds.

2. Experimental overview

The timespan of this experiment was two 4 h sessions, with a pre-lab quiz administered at the beginning of each session. The experiment was carried out in group of 3–4 students.

2.1. Natural product extraction

In the first session, students separated natural aldehydes from aromatic and medicinal plants (Figure 1) by steam distillation. The distillate was then extracted with ethyl acetate. The organic phases was gathered in a conical flask and dried by using anhydrous potassium carbonate or sodium sulphate. Filtration and evaporation of the solvent allowed obtaining the essential oils.(O'Shea et al., 2012)

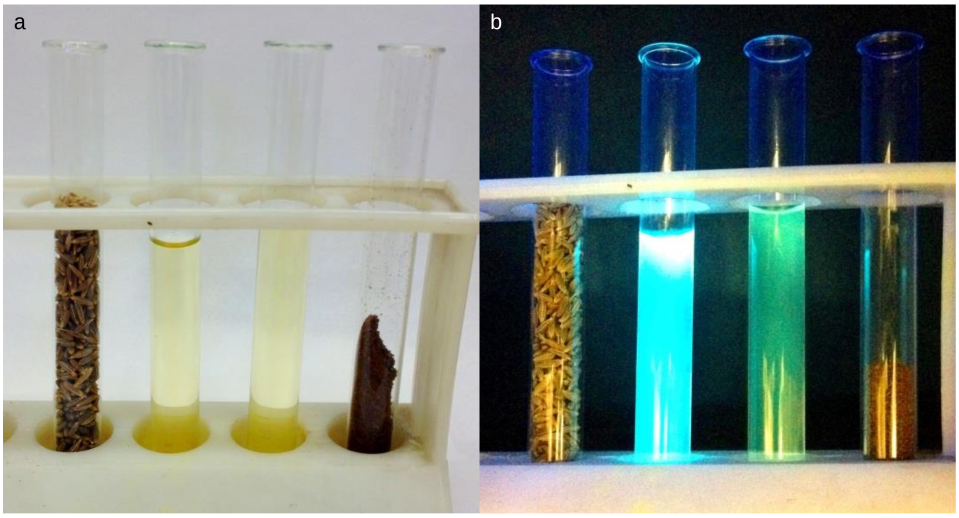


Fig. 1. a) From left to right: test tubes filled with cumin seed, fluorophore obtained from cumin aldehyde, fluorophore obtained from vanillin aldehyde, powdered vanilla seeds. b) The same test tubes under UV-lamp, despite the similar wavelength emission (478 nm for **1** and 489 nm for **2**), the perceived color is clearly distinguishable.

Essential oils are the more volatile substances found in roots, barks, leaves, flowers, fruits or seeds of plants. They have a wide range of applications, from medicine to flavoring agents and perfumes. Some examples are quinine from the bark of cinchona tree, vanillin from vanilla pods, and rose oil

from rose petals. In this paper we provide several common cooking spices as source of aromatic aldehydes. Traditionally, essential oils are steam distilled and then separated from water using a solvent. Even if essential oils are immiscible with water and do not required the use of organic solvents their separation, a solvent is usually employed to achieve a better recovery.
In choosing the plants for this experiment, different criteria were considered and preference was given to plants that students already have some familiarity with. In this paper we present vanillin and cumin aldehydes extraction and reaction, anyway many other plants can be used for the same experiment. An important requirement in choosing the plants was that the aldehyde could be obtained through steam distillation, without chromatographic purification, in large amount and with the minimum required purity to be characterized and used for the second step.(Azimova et al., 2012; Croteau, 1992; Franklin and Keyzer, 1962) To this end, cumin-aldehyde constitutes one of the major component of Cuminum cyminum essential oil and vanillin constitutes the primary component of the extract of the vanilla bean. Cumin is a very cheap and readily available spice, it provides cuminaldehyde easily and with high purity.(Bankar, 2011; Glidewell, 1991; Morshedi et al., 2015) Conversely, vanilla seeds are very expensive. A useful low-cost alternative, previously reported in literature, consists in the easy purification of the commercial vanilla alcoholic extract.(Ainscough and Brodie, 1990; Beckers, 2005; Cicchetti et al., 2010; Sinha et al., 2008)

2.2. Steam-distillation

In principle, steam distillation works by separating volatile, water-insoluble compounds from nonvolatile compounds through codistillation (See Scheme 3). The process is an application of Dalton's Law of Partial Pressures. The law tells that the total pressure (P_T) of the system is equal to the sum of the partial pressures (eq. 1). In this case the total pressure is equal to the vapor pressure of the water (P_{H2O}) added to the vapor pressure of the essential oil (P_{oil}).

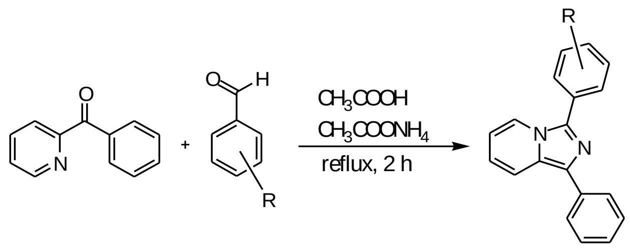
$$P_T = P_{H_2O} + P_{oil} \qquad (1)$$

While each essential oil has a unique vapor pressure, each contributes a few percent to the total vapor pressure. When the total vapor pressure above the solution is equal to atmospheric pressure the solution boils.
By steam distilling, the essential oil is collected at a temperature well below its own boiling point. Essential oils, while volatile, have boiling points well above the boiling point of water and, in many cases, their distillation results in the degradation of the desired product as it oxidizes at these high temperatures. Otherwise steam distillation significantly reduces the degradation.

2.3. Synthesis of fluorophores

In the second session, each group used the previously extracted aldehyde to synthesize a fluorophore (See Figure 1 and Scheme 3). The reaction involves the natural aldehyde, phenyl(pyridin-2-yl)methanone, and ammonium acetate in a one-pot cyclization approach.(Wang et al., 2005, 2003) As previously reported,(Volpi et al., 2016) 1,3-diarylated imidazo[1,5-a]pyridine derivatives can be synthesized in high yields with a one-step synthesis via condensation of phenyl(pyridin-2-yl)methanone with several aldehydes in presence of ammonium acetate (Scheme 1). In this work, in order to synthesize and study the photophysical properties of this class of fluorophores, two new 3-substituted-1-phenylimidazo[1,5-a]pyridines are presented; the preparation involved both vanillin aldehyde (from vanilla beans) and cumin aldehyde (from cumin) (Figure 2).

In general, this reaction is tolerant to various aldehydes and the optimal condition was established to have a 1:1,5:5 molar ratio of ketone/aldehyde/ammonium acetate.



Scheme 1. One pot synthesis of substituted 1-phenylimidazo[1,5-a]pyridines.

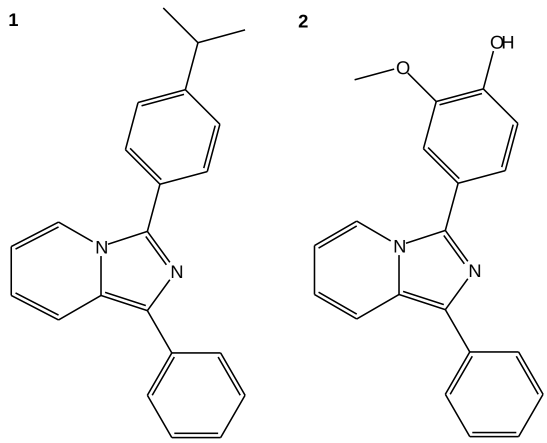
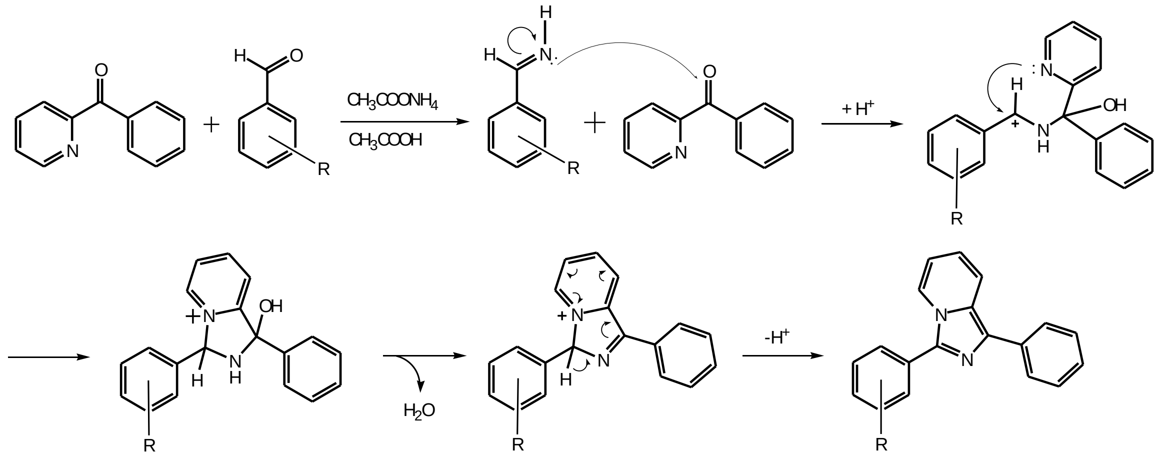


Fig. 2. 3-substituted-1-phenylimidazo[1,5-a]pyridines from cuminaldehyde (**1**) and from vanillin (**2**).

The reaction is very simple and was carried out without special precautions related to oxygen or water presence. Even in presence of impurities in the aldehyde extract, the synthesis led to the desired fluorescent product because of the high selectivity of the reaction between aldehyde and ketone. In any examined case we always observed an intense luminescence due to the reaction product. Moreover, the yellow color of the products and their intense luminescence highly help the visualization during TLC or chromatographic purification. A detailed description of the experimental procedure is reported in the Supporting Information.

The reaction mechanism involves the initial addition of ammonia to an aldehyde to form the corresponding imine, followed by the nucleophilic attack of the in situ generated imine to the carbonyl of the ketone (Scheme 2). These two steps bring the three individual components together. The consequent intramolecular cyclization leads to the 2-substituted-1-imidazo[1,5-a]pyridine. This experiment has been performed in an intermediate chemistry laboratory course with 28 enrolled students. Percent of students who successfully isolated, characterized, and correctly compared their molecule to a known standard (by TLC, ESI-MS, NMR): 85%. All students reported the correct appearance and instrumental results of the obtained essential oils and fluorescent products. 22 (of the 28 students) collecting yellow pure crystalline powder of the fluorescent product, and the remaining 6 students obtaining a yellow-brown solid or oil, likely due to impure distilled starting materials. All the students have observed the strong fluorescence in their solution products and have successfully characterized the fluorophores indicating the correct values of absorption and emission maxima in the final report.



Scheme 2. Synthetic reaction mechanism of substituted 1-phenylimidazo[1,5-a]pyridines.

2.4. Qualitative analysis

TLC was performed on TLC sheets coated with 0.25 mm layers of silica gel 60 F_{254}. Spots were visualized with a UV lamp. The sheets were developed in paper-lined all-glass chambers with 10 ml of dichloromethane-methanol (96-4), previously left to equilibrate for at least 10 min.
The distance to the center of the spot (d_{spot}) and the solvent (dichloromethane) front distance (d_{solv}) from the spot line are measured with a ruler. The Rf (retention factor) for the spot is calculated from $Rf = d_{spot}/d_{solv}$.
The TLC developing solvent efficiently separates cumin and vanillin aldehyde (Rf = 0.71 and Rf = 0.26 respectively in 100% CH_2Cl_2) and **1**, **2** (Rf = 0.32 and Rf = 0.09 respectively in 100% CH_2Cl_2).

Absorption and emission characterization

The imidazo[1,5-a]pyridine nucleus is well known in literature for its photophysical properties.(Garino et al., 2008; Ge et al., 2012; Hutt et al., 2012; Salassa et al., 2008; Volpi et al., 2017, 2016, 2009) Different absorption peaks fall in the wavelength range from 300 nm to 382 nm (as shoulders), while almost no absorption beyond 420 nm are observed/detected. The products show large Stokes' shifts (179 and 181 nm) and an intense fluorescent emission at wavelengths in the 470-490 nm range.
Absorption and emission spectra of compounds **1** and **2** in acetonitrile solution (~10^{-5} M) are presented in Figure 3, and their emission wavelengths are reported in Table 1. A qualitative characterization of the strong luminescence of the products is easily observable with a common UV lamp (Figure 1a).

Table 1. Photophysical properties of the obtained 1-phenylimidazo[1,5-a]pyridines in acetonitrile solution

Compound	λ_{abs} (nm)	λ_{em} (nm)	Stokes' shift (nm)
1	297 380[a]	478	181
2	310 380	489	179

[a]Shoulder.

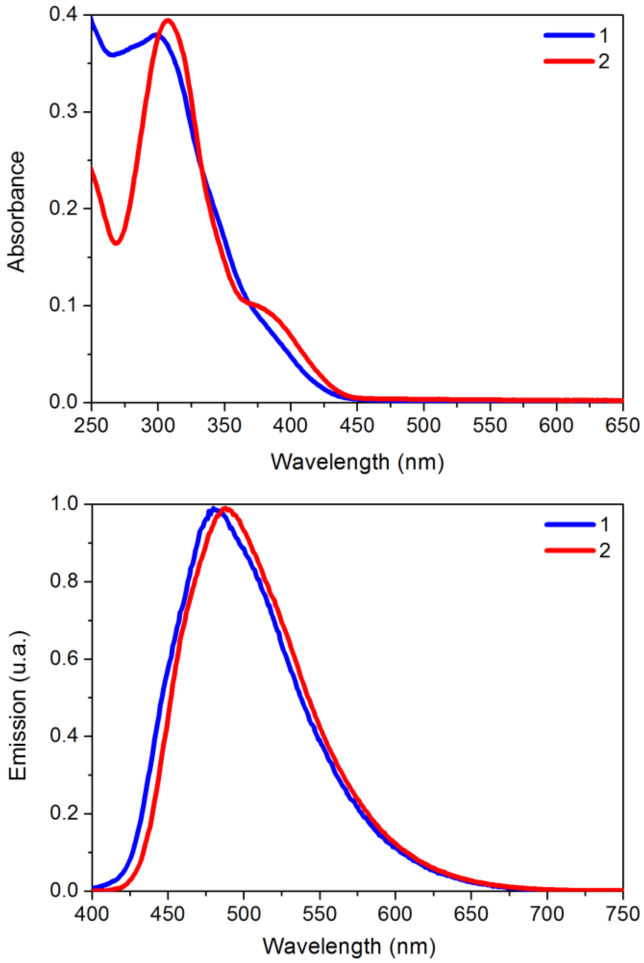


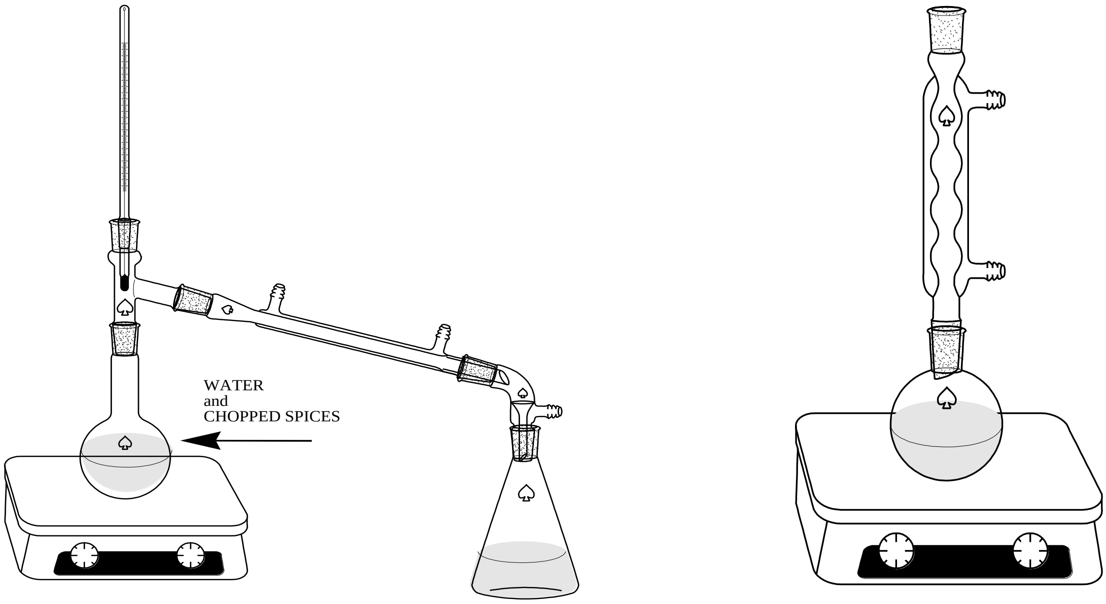
Fig. 3. UV-Visible absorption spectra (top) and normalized emission spectra (bottom) of compounds **1** and **2**, recorded in acetonitrile solution.

3. Hazards

Students must wear safety glasses and laboratory coats at all times. Steam distillation, extraction, synthesis and TLC analysis should be performed in a fumehood.

Ethyl acetate, acetonitrile and methanol are highly flammable, dichloromethane and methanol are toxic, the latter being particularly toxic when absorbed through the skin. Students are provided with access to the material safety data sheets for all chemicals, and are asked questions on safety aspects of the experiment as part of their pre-lab work. Acetic acid, ammonium acetate and phenyl(pyridin-2-yl)methanone are toxic. Avoid inhalation, contact with skin, especially near the eyes. Remember to wear safety glasses when preparing or presenting the experiment.

Essential oils have always been isolated from aromatic plants and both the feedstocks and essential oils commonly degrade in the environment. This is not to say that concentrated extracts are not dangerous to human health or the environment. All starting materials waste can be disposed in non-hazardous waste.



Scheme 3. Experimental setups: Steam distillation apparatus (left) and synthetic reflux system (right).

4. Conclusions

This laboratory experiment is appropriate for an intermediate, general or organic chemistry course. It involves simple, inexpensive starting materials, and demonstrates a number of important principles related to modern chemistry research.

The experiment had the following key learning outcomes:

- Improve the synthetic skills (extraction of a natural compound, solvent extraction, vacuum filtration, steam-distillation), assessed by yield and appearance of the extracts.
- Exposure to a range of analytical techniques (TLC, Mass spectrometry, fluorescence, NMR, absorption and emission spectroscopy), assessed by reported spectra.
- Improve data analysis (comparison between experimental and reported spectra).
- Improve understanding (for example, of the relationship between absorption-emission spectra and structure), assessed through preparation of the experimental report.

Students were able to observe that the identity structure of the natural aldehyde affects the photophysical behavior of the fluorescent product itself. Furthermore, students were required to compare measured spectra to literature values, which let them experience literature search.

In conclusion, in this experience it was possible to observe an example of extraction of organic compounds from common spices and an interesting one step cyclization reaction that has much potential for designing novel fluorescent molecules and probes.

At a somewhat more advanced level, other things which can be included in the discussion are the structures of the natural products as well as the corresponding fluorescent derivatives, and their absorption and emission of light, as students can properly understand the results.

While this experiment concern only vanillin and cumin aldehyde extraction and reaction, fluorescent products from other natural aldehydes have been previously reported and characterized. The paper is focused on vanillin and cumin, owing to their strong color changes and quantum yields of the products, anyway the experiment could readily be expanded to include other aromatic plants, essential oils and their derivatives (such as benzaldehyde from bitter almond oils, anisaldehyde from anise seeds, cinnamaldehyde from cinnamon).(Lee et al., 2014; Remaud et al., 1997; Taber and Weiss, 1998; Volpi, 2016; Walsh et al., 2012; Zheljazkov et al., 2013) Theoretical design of such compounds and investigation of some other systems into imidazo[1,5-a]pyridines for functional materials can be considered in choosing other aromatic plants or starting materials. These compounds represent an interesting class of fluorescent molecules easily tunable with potential interest in the fields of DSCs, OLED, NLO and for pharmaceutical applications. Moreover, high reaction yields, absence of catalysts, high accessibility and stability, ease of handling and preparation make this synthetic approach useful for student laboratory.

Supporting information

Experimental details, notes for instructor with complete characterization of **1**, **2** and essential oils, hazards, question and answer for lab experiences.

Acknowledgments

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