Aqueous Dearomatization/Diels-Alder Cascade to a Grandifloracin Precursor

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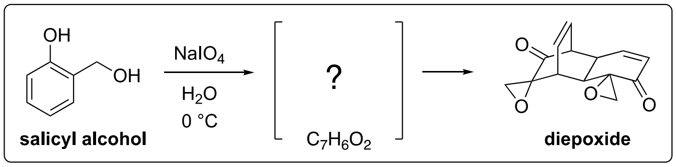
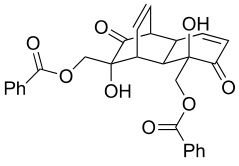
ABSTRACT

A green laboratory experiment has been developed in which students perform an aqueous oxidation/cycloaddition reaction to convert salicyl alcohol into a pentacyclic diepoxydione that is readily isolated by filtration. Drawing on their knowledge of periodate-mediated 1,2-diol cleavage, students propose a mechanism for the oxidation of salicyl alcohol (which is not a 1,2-diol) and the structure of the transient product (prior to a spontaneous Diels-Alder dimerization). Students then characterize salicyl alcohol and their diepoxide product by mass spectrometry, IR spectroscopy, and ^{1}H, ^{13}C, and two-dimensional NMR spectroscopy. The only organic solvents used are small amounts for IR and NMR spectroscopy.

GRAPHICAL ABSTRACT





grandifloracin

KEYWORDS

Second-Year Undergraduate, Upper-Division Undergraduate, Organic Chemistry, Inquiry-Based/Discovery Learning, Hands-On Learning/Manipulatives, Collaborative/Cooperative Learning, Green Chemistry, Phenols, Mechanisms of Reactions, NMR Spectroscopy.

INTRODUCTION

There is growing interest in avoiding organic solvents in laboratory reactions when possible,[1-3] and aqueous reactions represent a promising strategy to meet this goal in undergraduate experiments.[4-6] When the reaction product precipitates out of solution in relatively pure form, it may be possible to simply isolate the crude product without requiring additional solvent for extraction, recrystallization, or column chromatography.[7-8] Such is the case with the experiment described here, which also features a mechanistically interesting biomimetic reaction cascade that is a highlight of the recent racemic synthesis of the natural product grandifloracin (**1**, Figure 1) by Stoltz and coworkers[9] and was first reported by Adler.[10,11]

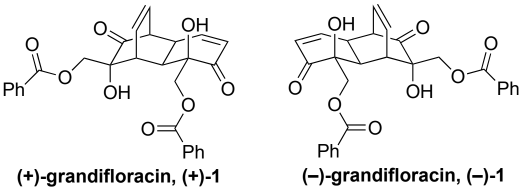
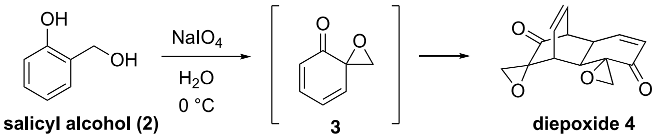


Figure 1. Structure of the plant-derived natural product grandifloracin.

Remarkably, both enantiomers of grandifloracin occur naturally in separate plant species. (–)-Grandifloracin was extracted from the stem and leaves of *Uvaria grandiflora* in 1997[12] and has no reported bioactivity. (+)-Grandifloracin was isolated from *Uvaria dac* in 2012[13] and has reported activity against pancreatic cancer cells.[13-14] Pancreatic cancer is an especially severe form of cancer, with low 5-year survival rates.[15] While the total synthesis of (±)-grandifloracin has not yet proven amenable to adoption in an undergraduate instructional laboratory, the key oxidative dearomatization of salicyl alcohol (**2**) into epoxyketone **3** and spontaneous Diels-Alder homodimerization to deliver diepoxide **4** (Scheme 1) is a highly selective and reliable reaction cascade that is well suited for intermediate and advanced undergraduates.

Scheme 1. Biomimetic cyclization cascade to form a grandifloracin precursor



PEDAGOGICAL SIGNIFICANCE

This experiment offers an excellent opportunity to improve students' skills in mechanistic reasoning and spectroscopic characterization. Students can apply their previous understanding of periodate-mediated glycol cleavage[16] to a related, but more challenging, context. For students who have seen Diels-Alder reactions previously, the cyclodimerization step can also receive emphasis.[17] The starting material (**2**) and product (**4**) in this experiment are strikingly different in their aqueous solubility as well as their IR, NMR, and mass spectra. A phenol and alcohol convert into ketones and epoxides, while the aromatic ring of **2** contrasts with the alkenes in **4**. Students and instructors alike may appreciate the biomimetic cascade and green chemistry aspects of this experiment (*e.g.*, use of water as solvent, modest scale, simple reaction conditions, rapid reaction time, ease of purification, striking increase in structural complexity, and minimal use of organic solvents).

The pedagogical goals of this experiment are for each student to:

- Propose a mechanism and product for a new reaction, reasoning by analogy from a known reaction
- Perform an aqueous, biomimetic reaction and isolate their product
- Characterize two different compounds using MS, IR, and several NMR techniques
- Collaborate effectively in a group for data collection and interpretation
- Appropriately tabulate their spectral data

EXPERIMENTAL OVERVIEW

The experiment described here took place during two 4-hour lab periods at the end of a first-semester organic laboratory, though this reaction has also been employed in second-semester and advanced organic laboratories. This experiment could be shortened to a single lab period (see instructor notes in the Supporting Information). On the first day, students submitted their answers to the pre-lab questions and performed the oxidation/dimerization reaction independently. Each student dissolved salicyl alcohol (100 mg) in water (2 mL), cooled the solution, and added sodium metaperiodate (286 mg). The reaction turned yellow-orange and was stirred in an ice bath for 30 minutes. A tan precipitate was isolated by vacuum filtration and air-dried for a week on a watch glass. While their product was drying, students collected MS, IR, and ^{1}H NMR data for salicyl alcohol as a group. The instructor provided additional spectra for **2** (^{13}C, COSY, NOESY, HSQC, and HMBC).

On the second day, students determined the mass and (unpurified) yield for their reactions and collected MS, IR, and ^{1}H NMR data for their product, again as a group. The instructor provided additional spectra (^{13}C, COSY, HSQC, and HMBC) and molecular models of salicyl alcohol (**2**) and diepoxide **4**. Students consulted the models and the instructor as they collaboratively analyzed all of the data for both compounds.

HAZARDS

Salicyl alcohol, water, and deuterium oxide are nonhazardous. Sodium metaperiodate is a strong oxidizer and can cause skin burns. Dimethyl sulfoxide-d_6 is a flammable liquid and is readily absorbed through the skin, potentially carrying dissolved chemicals into the bloodstream. Chloroform-*d* and dichloromethane are volatile, nonflammable liquids; chronic exposure can lead to an increased risk of developing cancer, in addition to adverse effects on the heart, central nervous system, and liver. Chloroform-*d* and dichloromethane can also cause skin and eye irritation. Diepoxide product **4** does not have known safety hazards, but should be treated as a potential skin and eye irritant. Gloves and protective eyewear should be worn for this experiment.

RESULTS AND DISCUSSION

This experiment was first tested by the authors and by a group of 10 first-year Harvey Mudd College (HMC) undergraduates in a research laboratory setting. It was then performed by nine second-semester organic students, followed by 14 first-semester organic students as their final laboratory experiment. Both sets of organic students were already familiar with MS and IR techniques, as well as ^{1}H, ^{13}C, and COSY NMR spectroscopy (including deuterium exchange). Some of the two-dimensional NMR experiments (NOESY, HSQC, and HMBC) were new to the students, so the instructor introduced the interpretation of these types of spectra in the laboratory. The experiment was also performed by 18 UCLA undergraduates in an advanced organic laboratory course as part of a longer synthesis.

All 51 students successfully prepared diepoxide **4**, with the first-semester organic students reporting yields of 21-100% (average yield = 61%, $N = 14$).[18] Even the student with the lowest yield (due to inadvertently spilling their product) had sufficient product for analysis. Students collaboratively collected ^{1}H NMR, IR, and MS data for **2** and **4**, and the product spectra were sufficiently clean to not require further purification beyond simple filtration. IR spectroscopy was conveniently performed using an ATR accessory (see Supporting Information page S26), and the authors found that superior IR spectra for salicyl alcohol—including two distinct, broad O-H absorbances at 3420 and 3160 cm^{-1}—were obtained from a thin film using dichloromethane rather than directly from the solid. The O-H peaks were notably missing in the IR spectrum of **4**, while two distinct C=O peaks appeared at 1735 and 1692 cm^{-1}. Atmospheric-pressure chemical ionization mass spectrometry (APCI-MS) of **4** gave prominent peaks for $[M+H]^+$ and $[M/2 + H]^+$ at 245 and 123 *m/z*, respectively—the latter peak represents a thermally induced retro Diels-Alder reaction in the spectrometer (see Supporting Information page S27). A diagnostic peak for $[M + H – H_2O]^+$ at 107 *m/z* was observed in the APCI mass spectrum for **2**. GC-MS was unsuccessful for **4** but gave peaks for **2** at 124 and 106 *m/z* ($[M]^{+\bullet}$ and $[M – H_2O]^{+\bullet}$, respectively).

Students were able to unambiguously assign all of the ^1H and ^{13}C signals in **2**, with the exception of two overlapping hydrogens at 6.76 ppm and two nearly coincident carbons at 127.3 ppm. Notable features of the ^1H NMR spectrum for **2** include a phenolic hydrogen at 9.28 ppm, four aromatic hydrogens, and clear coupling between the benzylic alcohol and benzylic hydrogens (see Supporting Information page S14). A D_2O shake removes this coupling and the two exchangeable alcohol signals. The NOESY spectrum indicates that the benzylic alcohol is oriented towards the phenol, enabling an intramolecular hydrogen bond. The HSQC and HMBC spectra helped students assign most of the other signals. Students gained confidence through interpreting the spectra for salicyl alcohol in advance of tackling the more difficult diepoxide **4**.

The ^1H NMR spectrum for **4** features four alkene signals—one doublet and three doublets of doublets (see Supporting Information page S20). The coupling patterns in the COSY spectrum help to identify these signals and the allylic hydrogens. The four epoxide hydrogens are all sharp doublets that couple in pairs with J values of 6 Hz. The HMBC spectrum allows differentiation of the carbonyl carbons as well as the epoxides, though the diastereotopic epoxide hydrogens are not easily differentiated without further information (a NOESY spectrum did not significantly clarify this matter and so was not included in the experiment).

Students' articulations of predicted differences between **2** and **4** in the IR, MS, and ^1H NMR data, respectively, were correct in 100, 93, and 86% of the pre-lab assignments submitted. An impressive 93% of students correctly predicted the oxidation mechanism. Further evaluations of student learning outcomes are listed in Table 1. Overall, student performance was acceptable for all of the tasks assessed, with the most difficulty encountered in correctly assigning NMR signals for **4** (not surprisingly) and in clearly tabulating all of the spectral data (which requires discipline and patience). An additional, implicit goal for this experiment was for students to appreciate aspects of green chemistry, an explicit theme of prior experiments in the semester. Disappointingly, only one out of 14 students, unprompted, noted any of the environmentally friendly aspects of this experiment in their laboratory notebook. This observation underscores the importance of continuing to feature green chemistry prominently and of specifically directing students to reflect on sustainability concepts if instructors desire students to be mindful of these themes. As a result, specific questions about green chemistry were added to the pre-lab questions and the lab write-up instructions. Sample calculations for atom economy and reaction mass efficiency[19] have been added to the instructor notes, and the student handouts have been revised to be usable by both first- and second-semester organic students. All of these may be found in the Supporting Information.

Table 1. Student Learning Outcomes

Learning Objective	Students Should Be Able To:	Students[a] Successfully Performing Task, %
1	Obtain desired product	100[b]
2	Predict oxidation mechanism	93
3	Predict structure of intermediate **3**	86
4	Correctly assign ^{1}H and ^{13}C NMR signals for **2**	100
5	Correctly assign ^{1}H and ^{13}C NMR signals for **4**	78
6	Correctly assign IR signals for **2** and **4**	86
7	Correctly assign MS signals for **2** and **4**	86
8	Collaborate effectively for data collection and interpretation	100
9	Clearly tabulate all spectral data for **2** and **4**	78

[a] N = 14. [b] N = 51 for objective 1 only.

CONCLUSIONS

In this experiment, undergraduate organic students proposed the mechanism and product for an oxidative dearomatization reaction, performed a tandem oxidation/Diels-Alder reaction in water, isolated their product, and collected MS, IR, and NMR data collaboratively to characterize their starting material and product. The experiment challenged all students with pre-lab problem solving, spectral predictions, and spectral analysis for both simple and complex molecules. Students responded well to the collaborative environment, but they largely neglected to address green chemistry aspects of the experiment when not explicitly asked to do so. To demonstrate the portability of this experiment to other institutions, 18 undergraduate students in an advanced organic laboratory course at UCLA performed this reaction; all of the students successfully obtained the desired product and addressed mechanistic questions similar to those posed for the Harvey Mudd students.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.XXXXXXX. [ACS will fill this in.]

Student handout, instructor notes with list of chemicals, representative student spectra (PDF, DOCX)

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17. The revised student handouts in the Supporting Information have questions addressing the transition state and regioselectivity of the Diels-Alder reaction in the lab write-up section.
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