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Laboratory Experiment

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# Detection of Salicylic Acid in Willow Bark: An Addition to a Classic Series of Experiments in the Introductory Organic Chemistry Laboratory

Matthew D. Clay* and Eric J. McLeod

Department of Chemistry, St. Mary's University College, Calgary, AB, Canada, T2X 1Z4

*Supporting Information*

**ABSTRACT:** Salicylic acid and its derivative, acetylsalicylic acid, are often encountered in introductory organic chemistry experiments, and mention is often made that salicylic acid was originally isolated from the bark of the willow tree. This biological connection, however, is typically not further pursued, leaving students with an impression that biology and chemistry are separate entities in the laboratory. We have developed a laboratory that investigates this biological connection in which salicylic acid is extracted from willow bark and detected using thin-layer chromatography. This experiment can be adopted as either a standalone experiment or integrated into other laboratory courses with current experiments involving salicylic acid.


**KEYWORDS:** *First-Year Undergraduate/General, Second-Year Undergraduate, Interdisciplinary/Multidisciplinary, Laboratory Instruction, Organic Chemistry, Hands-On Learning/Manipulatives, Drugs/Pharmaceuticals, Natural Products, Thin Layer Chromatography*

The widespread use of acetylsalicylic acid (Aspirin) and its relatively simple structure make it an attractive synthetic target in the introductory organic chemistry laboratory, and a number of effective syntheses for undergraduate laboratories have been developed.[1] Many laboratory courses also include an experiment wherein thin-layer chromatography (TLC) is used to assess the purity of the synthesized acetylsalicylic acid and to analyze the composition of various commercial analgesic preparations.[2,3] In both experiments, the introductory matter often includes a discussion of the history of salicylic acid, focusing on the use of willow bark in native medicine to relieve fever and pain and the subsequent discovery that the therapeutic properties of the bark were due to salicylic acid.

The biological connection of acetylsalicylic acid to willow bark, however, is not further pursued experimentally, which likely reinforces the mistaken belief held by students that biology and chemistry are separate entities,[4] particularly within the laboratory. Not only may this misconception be detrimental to those students entering an increasingly multidisciplinary scientific community,[5] but those students whose interests lie in biology (for which organic chemistry is often a required course) may be missing some of the important connections to their discipline that could serve to motivate them and improve their view on the relevance of the course.

We have developed an experiment for the introductory organic chemistry laboratory in which salicylic acid is extracted from willow bark and detected using TLC. This experiment can be run by itself in one 3-h laboratory session or easily integrated into the classic experimental sequences involving salicylic acid referenced above. Students are introduced to the common experimental techniques of extraction, TLC, and the use of a reflux apparatus, while the modern reality that the chemical laboratory is highly interdisciplinary is reinforced.

The detection of salicylic acid in willow bark is not a novel idea. The rising popularity of willow bark extract as an alternative therapeutic to acetylsalicylic acid has led to the development of many effective methods for the detection and quantification of salicylic acid and its derivatives in willow bark.[6,7] However, the techniques used are not suitable or easily adapted to the introductory organic chemistry laboratory, necessitating the development of a method for the rapid extraction and detection of salicylic acid.

## ■ EXPERIMENTAL DETAILS

The experiment consists of three parts that can be completed in a typical 3-h laboratory session by pairs of students. Students first process the willow branches, separating the bark from the leaves, wood, and other components of the branch and then reflux the bark in hydrochloric acid. Finally, extraction of the mixture with ethyl acetate yields a crude extract that can be analyzed by TLC for the presence of salicylic acid.

### Collection and Preparation of Willow Bark

Willow branches used in this experiment can be collected from willow trees in backyards, parks, or wilderness areas. Five species of willow (*Salix fragilis*, *Salix amygdaloides*, *Salix*

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*tameline*, *Salix bebbiana*, and *Salix pentandra*) were tested and all gave satisfactory results. Young branches ~4−8 mm in diameter and ~30 cm in length may either be harvested from living trees or collected from the ground (use only freshly fallen branches having a green inner bark). No problems were noted using branches that were either freshly collected or that had been stored at −20 °C for 1 year. Five to seven branches of this size will provide ample bark for the experiment per pair of students. As it is known that the quantity of salicylates in willow bark can fluctuate depending on the season,[7] care should be taken to test branches from the same season in which the laboratory will run. No difference was found between samples from both summer and early fall for the purposes of this experiment.

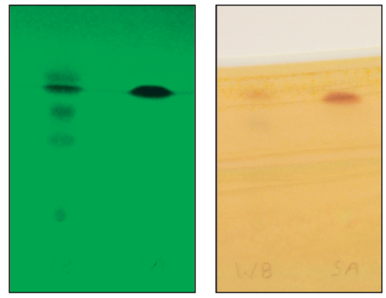
Students were provided with the unprocessed branches (or collected branches themselves) and peeled the bark from the branches using their fingers or a utility knife. Both the hard outer bark and softer green inner bark were collected as both contain salicylic acid and its derivatives; large woody parts cut off by the knife were discarded. For practical purposes, the bark was cut into small pieces so that it fit conveniently in a 100 mL round-bottom flask; 10−15 g of bark were used per experiment.

### Hydrolysis of Salicylates and Extraction of Salicylic Acid

Willow bark contains very little free salicylic acid as the majority exists in the form of salicin and other derivatives, primarily esters. Although the greatest quantity of salicylic acid could be obtained from salicin, a *simple* procedure to convert it to salicylic acid that would incorporate well into the curriculum was not devised. As this experiment involves the qualitative detection of salicylic acid, the bark was refluxed in 1.0 M HCl for 30 min to hydrolyze any ester derivatives (salicylates) present and ensure sufficient salicylic acid was present for TLC analysis. During reflux, the bark and acid became brilliantly red. After separation of the bark from the acid solution, the acid was extracted twice with ethyl acetate to yield a brightly colored crude extract that was decolorized with charcoal. This extract can be stored in the freezer (−20 °C) for at least one year without detectable change.

### Thin-Layer Chromatographic Analysis of Extract

To analyze the crude extract by TLC, a solvent system consisting of toluene, diethyl ether, glacial acetic acid, and methanol in a 120:60:18:5 volume ratio worked well.[3] An authentic sample of salicylic acid was run as a co-sample. Two methods were used to visualize the chromatogram. Ultraviolet radiation revealed several spots ranging in retention factor and intensity, but the spot for salicylic acid was the most intense. In addition, 1% ethanolic ferric chloride was used to selectively stain phenols present in the extract. Salicylic acid was the major component visible on the TLC using this method, with only one other phenol being occasionally observed. Examples of typical student chromatograms developed using both methods are provided in Figure 1.



A B

**Figure 1.** (A) UV−visualized and (B) $FeCl_3$-stained chromatograms of the willow bark extract from *S. pentandra* (WB = willow bark extract; SA = salicylic acid standard).

## ■ HAZARDS

Students employ 1.0 M hydrochloric acid in this experiment. Hydrochloric acid is corrosive and all spills should be treated with sodium bicarbonate. Gloves should be worn to limit the risk of skin contact when working with hydrochloric acid. Areas of accidental skin contact should be washed for at least 15 min with cool water; eyes should be flushed at an approved eyewash station for at least 15 min, and in both cases prompt medical attention should be sought. The organic solvents used in this experiment (ethyl acetate in the extraction and toluene, acetic acid, methanol, and diethyl ether in the TLC eluent) are all potentially harmful. All work involving the TLC eluent should be done in a fumehood. In case of inhalation of any solvent, remove to fresh air and seek medical attention. Areas of skin or eye contact should be treated as described above for hydrochloric acid. Students allergic to aspirin should take additional safety precautions to limit their exposure to willow bark and the extract.

## ■ RESULTS AND FEEDBACK

This laboratory has been run during the previous two fall semesters with a total of 18 students each year working in groups of two in a 3-h session. Procedural changes between year one and two, primarily the use of ethyl acetate as solvent instead of dichloromethane and the inclusion of a decolorization step using activated carbon, did not significantly affect the outcome of the experiment. The hydrolysis and extraction occurred without difficulty, and the brilliant red color that formed during the extraction was appreciated by students who had become accustomed to colorless solutions and white crystals in organic chemistry.

Student feedback was assessed in the second year of this experiment (14 respondents) via an anonymous survey using a standard five-point Likert scale. The results of the survey are summarized in Table 1. Although the sample size is small, it is apparent that students had a very positive view of the experiment, with nearly all strongly agreeing that it should be continued in future years. Of particular interest was the response to question 3, which indicated that students felt this laboratory illustrated the interconnectedness of biology and chemistry, and to question 6, which indicated that students generally appreciated interdisciplinary laboratory experiments of this nature.

## ■ INCORPORATION INTO CURRICULUM

This laboratory experiment can be incorporated into the introductory organic chemistry curriculum with relative ease, either as a self-contained experiment or connected to other experiments involving salicylic acid. We have had great success with the latter, integrating this experiment into a three-week, three-session sequence (each of 3 h): in session one, students synthesized acetylsalicylic acid (and acetaminophen); in session two, the crude willow bark extract was prepared; and in session three, TLC was used to analyze both samples, as well as the composition of commercial analgesics. With this sequence, new techniques were introduced and reinforced at an appropriate

**Table 1. Student Feedback**

| Question | Number of Responses[a] SD | D | N | A | SA | Ave[b] |
|---|---|---|---|---|---|---|
| 1. The experiment taught me new and valuable laboratory skills. | - | - | 2 | 8 | 4 | 4.14 |
| 2. The experimental procedure was straightforward and easy to carry out. | - | - | 1 | 9 | 4 | 4.21 |
| 3. The experiment increased my understanding of the interdependence of biology and chemistry. | - | - | - | 7 | 7 | 4.50 |
| 4. I have talked positively about this experiment to friends and family. | 1 | - | 1 | 4 | 8 | 4.29 |
| 5. The lab was too simple and required little thought. | 2 | 9 | 3 | - | - | 2.07 |
| 6. I value the inclusion of a biological component in the chemistry lab curriculum. | - | - | - | 6 | 8 | 4.57 |
| 7. The experiment should be continued in future years. | - | - | - | 2 | 12 | 4.86 |

[a]SD = strongly disagree; D = disagree; N = neutral; A = agree; SA = strongly agree. [b]The average was calculated by assigning numbers to the responses with strongly disagree assigned to 1 and strongly agree assigned 5.

rate for an introductory organic chemistry class. The experiment was also attractive from a financial perspective, requiring no nonstandard equipment and only inexpensive consumables.

The widespread distribution of willow trees in North America should also make this experiment adaptable to many institutions, but the ease of access to willows should be considered if students will be expected to collect their own branches. Those collecting should also be aware of local regulations as many urban parks forbid the collection of live specimens, although fewer problems should be encountered if only fallen branches are sought.

The possibility also exists that this laboratory can be connected to biology classes running concurrently with organic chemistry. We are currently collaborating with a professor of biology to develop a laboratory in ecology in which students will study the community ecology of willows and learn to identify them just prior to conducting the experiment in organic chemistry, thus, permitting students to confidently collect their own willow branches. This continuity between their classes will serve to illustrate the significant ties between the two disciplines.

## ■ CONCLUSION

This experiment expands on the historical and biological aspects of salicylic acid, making these connections in the laboratory rather than in text, and complements experiments already in place at many institutions. The introduction and reinforcement of many key experimental techniques in organic chemistry make this experiment highly relevant to the chemist, while its interdisciplinary nature will increase its appeal to a broad range of students with interests in areas other than pure chemistry.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Student handout and notes for the instructor. This material is available via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION


### Corresponding Author

*E-mail: matthew.clay@stmu.ca.


### Notes

The authors declare no competing financial interest.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