Understanding the Uniqueness of Artocarpus Flavonoids: Isolation and Structure Elucidation of Cycloartocarpin from the Roots of Artocarpus altilis

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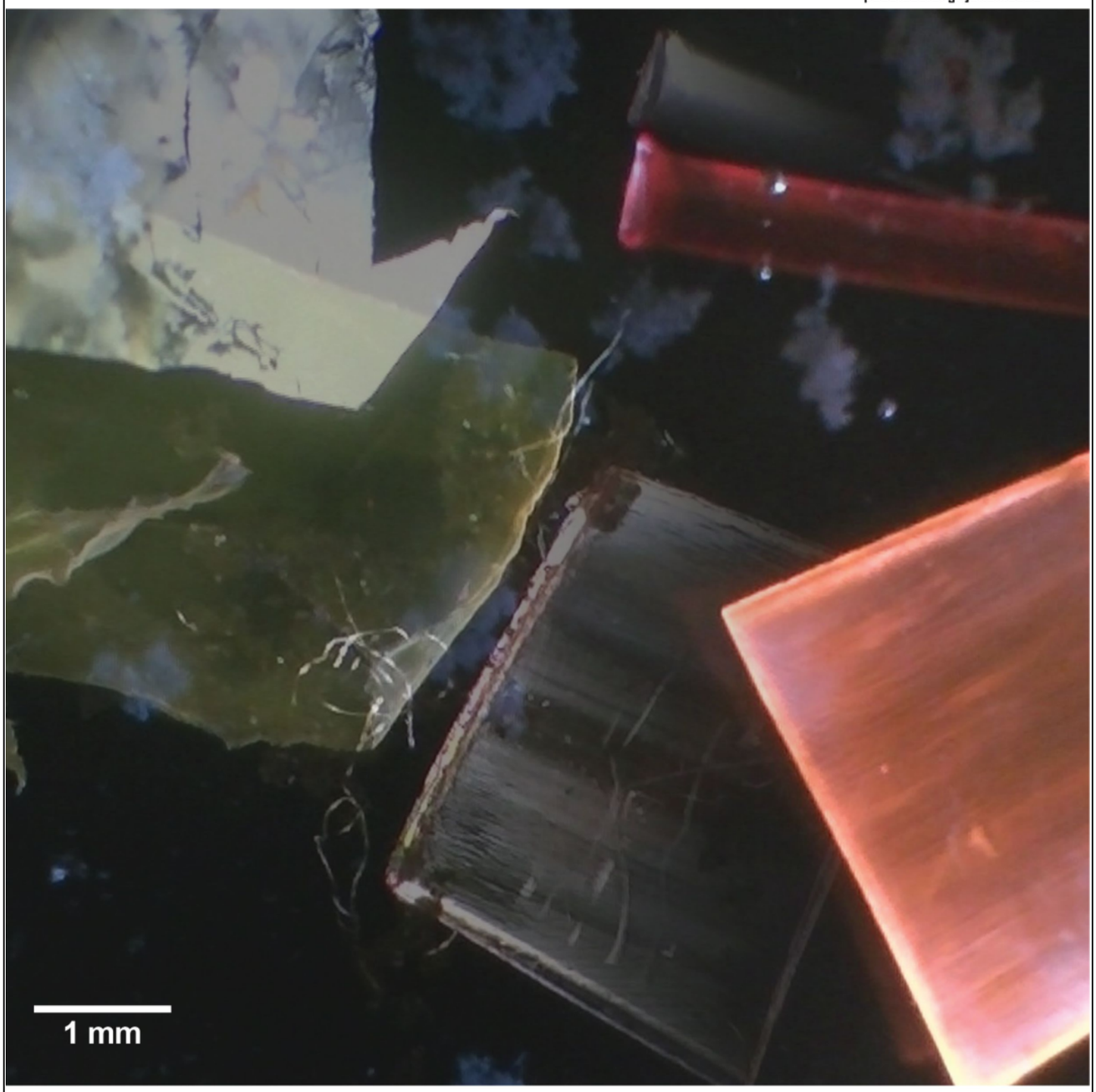

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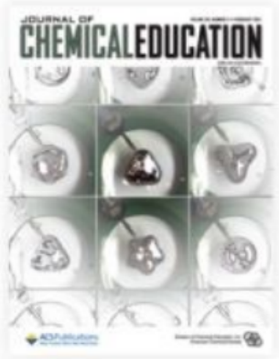

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Laboratory Experiment

Understanding the Uniqueness of Artocarpus Flavonoids: Isolation and Structure Elucidation of Cycloartocarpin from the Roots of *Artocarpus altilis*

Aliefman Hakim,* A. Wahab Jufri, Jamaluddin, Supriadi, and Putri Ayu Mutmainnah

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ABSTRACT: Secondary metabolite compounds contained in each plant genus have certain characteristics, which is true also of the flavonoid in the genus *Artocarpus*. In the experiment described here, third-year undergraduate students performed the isolation and characterization of cycloartocarpin from the roots of *Artocarpus altilis* (commonly known as breadfruit), giving them experience in skills such as extraction, fractionation, purification, and the structural elucidation of cycloartocarpin. Manipulation of the liquor by thin-layer and gravitation column chromatographic techniques proved to be a simple method to isolate cycloartocarpin. The flavonoid structure, cycloartocarpin, was treated with structure elucidation 1- and 2-dimensional NMR and infrared spectrometry. Students then compared the structure of the cycloartocarpin obtained with that of other flavonoids from the genus *Artocarpus* and other plant genera. From these comparisons, they were able to formulate structural characteristics of the flavonoid compounds of the genus *Artocarpus*. The laboratory method, accomplished in four, 3 h laboratory sessions, allowed students to readily understand the process of isolating flavonoids and their peculiarities.

KEYWORDS: *Upper-Division Undergraduate, Organic Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Problem Solving/Decision Making, Natural Products*

INTRODUCTION

The purpose of this laboratory was first to provide students with the essential skills required to perform the extraction, fractionation, purification, and structural elucidation of a flavonoid. Second, it provided them with knowledge of how to formulate the structural characteristics of secondary metabolites from a plant genus, in this case, the flavonoids from the genus *Artocarpus*. Many of the activities in this laboratory may be appropriate for other courses, such as ones involving the structure elucidation of organic compounds, organic chemistry, and chemotaxonomy.

Secondary metabolites, including flavonoids from the genus *Artocarpus* are isolated by extraction, fractionation, purification, and structural elucidation.[1,2] Furthermore, students can formulate the structural characteristics of the flavonoids from the genus *Artocarpus* by comparing their structure with those of other flavonoids from the genus *Artocarpus* and from other plant genera. These activities were carried out in this natural product chemistry laboratory. The natural product chemistry laboratory had been previously described in several studies.[2−10]

Several studies on the genus *Artocarpus* have shown that plants from this genus contain phenol derivative compounds, especially in flavonoid groups that display certain peculiarites.[11,12] The peculiarities involve the prenylated group in position C-3 and ring B oxygenated at position C-4′ or C-2′, C-4′ or C-2′, C-4′, C-5′. In addition, prenylation can also occur at positions C-6 and C-8; such a pattern is not found in other genera (Figure 1). The the flavonoid structure in the genus *Artocarpus* is unique, and the flavonoid displays interesting biological activities, such as antimalarial,[13] anticancer,[14] and cytotoxic[15] ones.

The structure of flavonoids from other plant genera follows the shikimic acid and acetic malonate biogenesis pathways. Comparison between the flavonoid prenylation and oxidation of the genus *Artocarpus* and other plant genera can be seen in Figure 1.

METHODOLOGY

This study uses quasi-experimental research with one group pretest−posttest design. Participants in this study consisted of 32 third-year students (teacher preparation) from chemistry education department at one of the state universities in West Nusa Tenggara, Indonesia. These laboratory activities took place during the second semester of the 2018−2019 academic years. Participants were divided into eight groups with four participants per group. All of the groups worked on the same plant sample, roots of *Artocarpus altilis* (commonly known as breadfruit).

A questionnaire containing 15 questions was used to assess the student knowledge of isolation processes and the ability of students to analyze the uniqueness of *Artocarpus* flavonoids.

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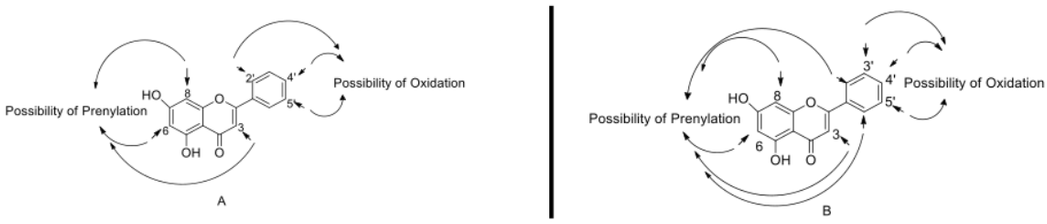

A. Prenylation and oxidation pattern of genus *Artocarpus* flavonoids.
B. Prenylation and oxidation patterns of flavonoid in other genera.

Figure 1. Possibility of prenylation and possibility of oxidation pattern of flavonoids.

The validity of the test was determined by using content validity experts. Qualitative analysis was done by interpretive descriptive analysis. Quantitative analysis of data was done by calculating the percentage of normalized gain scores (%g) using the formula in eq 1.[16]

$$\%g = \frac{S_{post} - S_{pre}}{S_{max} - S_{pre}} 100 \quad (1)$$

where S_{post} and S_{pre} are the post-test and pretest scores, respectively, and S_{max} is the maximum possible score. Values of %g were then characterized as high for $\%g > 70\%$, medium for $30\% \leq \%g \leq 70\%$, and poor for $\%g < 30\%$.

Laboratory reports were also used at the end of the laboratory activity that was arranged in groups. Student surveys were used to demonstrate student engagement with the experiment.

EXPERIMENTAL PROCEDURE

The experiment, conducted over four, 3 h laboratory sessions, was useful for the third-year undergraduate students. Throughout the experiment, they gained first-hand experience of various techniques involved in the extraction, isolation, and purification of cycloartocarpin, a flavonoid compound, from *Artocarpus altilis* roots. The students were able to formulate the unique structure of the genus *Artocarpus* flavonoid by comparing the structure of cycloartocarpin obtained with that of other flavonoids from the genus *Artocarpus* and other plant genera. Procedures of the student activity are described below; detailed procedures can be found in the Supporting Information.

Isolation of Cycloartocarpin from *Artocarpus altilis* Roots

The cycloartocarpin isolation procedure conducted in this laboratory activity differs from previous descriptions in the literature.[17,18] Students followed these steps for the first week:

- The groups prepared air-dried root stems of *A. altilis* by macerating these using methanol.
- The methanol extract was filtered using cellulose filter paper.
- The solvent was evaporated by a rotary evaporator to dryness to obtain a crude extract.

During the second week of the experiment, students undertook these steps:

- The crude extract was fractionated using gravity column chromatography.
- A single compound was characterized by the presence of a single spot in a TLC chromatogram.

This isolation procedure was also carried out by students in the previous semester, the second semester of 2017−2018. The isolation procedures were used by the students two times. Further details are provided in the student handout of the Supporting Information.

Structure Elucidation Studies

In the third week of the experiment, students performed IR and detailed NMR spectroscopic analyses and confirmed that the substances isolated were cycloartocarpin. The IR (KBr) spectrum of V_{max} (cm^{-1}) showed the presence of a conjugated carbonyl group (C═O), typical for flavone at an uptake of 1651 cm^{-1} and C═C aromatic groups at an uptake of 1620−1450 cm^{-1}. The IR spectrum also showed the presence of absorption for the hydroxyl (OH) group at V_{max} = 3390 cm^{-1} and aliphatic C−H at V_{max} = 2924−2854 cm^{-1}. The IR data of a single compound showed that all functional groups were found in the cycloartocarpin compounds, as seen in Figure 2.

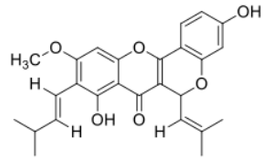

Figure 2. Structure of cycloartocarpin.

Further determination of the structure of a single compound was obtained from the ^{13}C NMR, which indicated 26 signals of carbon atoms. There was a chemical shift at δc 178.4 ppm, which is a typical sign of carbonyl (C═O), and a sign of carbon oxyaryl at δc(139−162) ppm. In addition, there were also signs of aromatic carbon at δc 104−125 ppm, including four methyl carbons and carbon methoxy. ^1H NMR (500 MHz, $CDCl_3$) showed a typical sign in the region of 13.42 ppm for a chelated −OH, which was evidence of flavonoid derivative. The pirano group γ,γ-dimethyl alyl was indicated by the presence of proton signals in chemical shifts at 1.69 (3H, s), 1.96 ppm (3H, s); 5.43 ppm (1H, d, J = 9.4 Hz); 6.25 ppm (1H, d, J = 9.4 Hz). Proton signals for ring B were shown at chemical shifts of 6.42 ppm (1H, d, J = 2.3 Hz); 6.52 (1H, dd, J = 2.3; 8.5 Hz); 7.66 (1H, d, J = 8.5 Hz). These signals indicate the existence of the ABX system on ring B, which was substituted at positions 2′ and 4′. The signal for the proton in the methoxy group was in the 3.93 (3H, s, OCH_3) chemical shift. HSQC showed five C-oxiaryl signals, six C-quaternary signals, and nine signals, including four methyl, methoxyl, and

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carbonyl. HMBC showed that ring A was substituted by a prenyl group substituted at C-6 in the presence of proton correlation of chelated OH with carbon from the prenyl group. More details can be found in the Instructor Notes of the Supporting Information.

Formulation of the Uniqueness of the Flavonoid Structure of the Genus *Artocarpus*

In the fourth week, students conducted a literature study through the internet and university library in order to find the structure of flavonoid compounds from the genus *Artocarpus* and other plant genera. Furthermore, they compared the patterns of prenylation and oxidation of the structure of isolated compounds with the structure of the flavonoid compounds obtained from the literature. From this activity, the students were able to deduce the unique flavonoid structure of the genus *Artocarpus*. The complete assignment is provided in the Supporting Information. Following completion of the experiment, each group of students submitted a detailed report.

Students Result

Of the eight groups who participated in this laboratory, only three groups succeeded in getting a single compound (based on the purity of TLC chromatograms compared with standard cycloartocarpin). However, all groups were still given the IR and NMR spectra provided by the instructor for interpretation by all groups. From the reports collected, all groups could determine all functional groups found in the cycloartocarpin compounds based on IR data. However, they had difficulty interpreting the NMR spectrum. Two groups could show an almost-correct interpretation of the NMR data. The other groups demonstrated poor NMR interpretation abilities. Six groups succeeded in formulating the specificity of *Artocarpus* flavonoids in the conclusions of their laboratory report. In the quantitative analysis of pretest and post-test scores, the average of students' n-gain percentage was 63% (medium category). See the student gain score spreadsheet in the Supporting Information.

HAZARDS

This experiment uses some potentially hazardous substances and flammable solvents, including methanol, dichloromethane, *n*-pentane, and petroleum ether (highly flammable). The use of flammable solvents should not involve direct flames. In addition, dichloromethane has limited evidence of a carcinogenic effect. The use of carcinogenic substances must be carried out in a fume hood. Ultraviolet (UV) radiation can cause severe damage to the eyes, so one should not look directly into the light source. Students should use personal protective equipment, including gloves and safety goggles.

SUMMARY

This experiment, with its straightforward method, allows students to isolate and characterize cycloartocarpin, an *Artocarpus altilis* flavonoid compound, and understand its uniqueness over four, 3 h laboratory sessions. Students work in small groups. If one group fails to isolate the pure material, that group can join a successful group for subsequent characterization and analysis. Isolated compounds can be replaced and come from different genera.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.0c00199.

Instructor notes (PDF, DOCX)

Student handout (PDF, DOCX)

Student gain score spreadsheet (XLSX)

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Notes

The authors declare no competing financial interest.

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