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by Wahab Jufri

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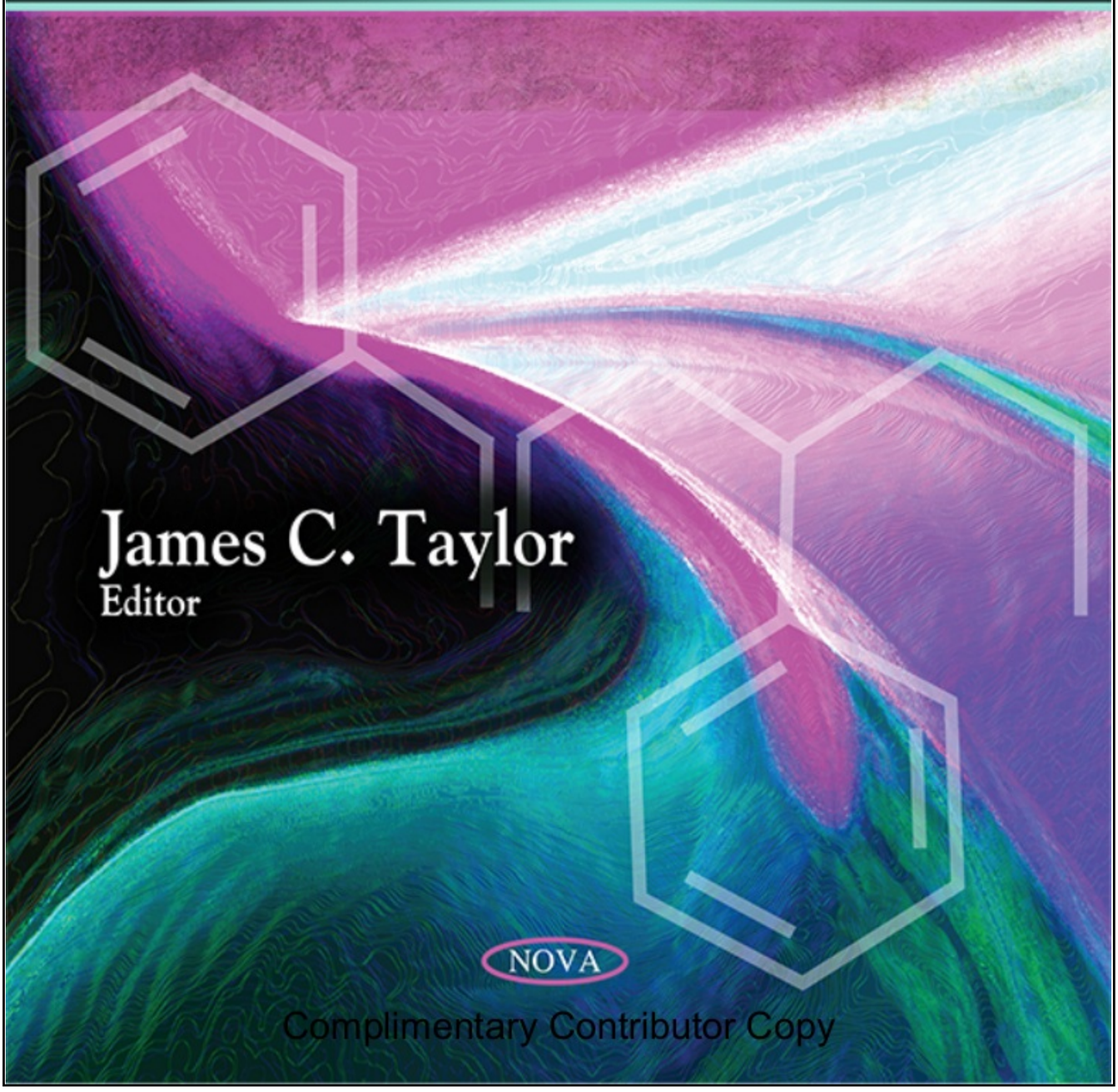

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Chapter 9

APPLICATIONS OF ISOLATION AND STRUCTURE ELUCIDATION OF SECONDARY METABOLITES IN NATURAL PRODUCT CHEMISTRY LABORATORY

Aliefman Hakim[1] and A. Wahab Jufri[2]
[1]Study Program of Chemistry Education, University of Mataram, Mataram-Lombok, Indonesia
[2]Study Program of Biology Education, University of Mataram, Mataram-Lombok, Indonesia

ABSTRACT

Natural products chemistry examines secondary metabolites contained in an organism, so it is strongly associated with pharmaceuticals, cosmetics, and pesticides. The chemical study of natural product based on experimental development and applications demanding high standards of laboratory activities. The laboratory activities involve the isolation of secondary metabolites from plants. The same secondary metabolites from a plant species can be isolated in a various ways, so there is no standard procedure to isolate the secondary metabolites of a plant species. These conditions can be used to train high-level thinking skills of learners. In natural product chemitry laboratory, learners can be given responsibility to undertake project to isolates the secondary metabolites from a variety of plant species. This laboratory works provide

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opportunities for students to design their own activities to isolate the secondary metabolites from medicinal plants. Students are exposed to skills as extraction, fractionation, purification, and structural elucidation of secondary metabolites. These laboratory activities can be useful for students at the third-year undergraduate level from many different disciplines including chemistry education, chemistry, pharmacy, and medicine.

Keywords: natural products, secondary metabolites, isolation, structure elucidation, laboratory

INTRODUCTION

Humanity is dependent on nature. People can obtain food, medicines, building materials, and other resources from the nature. Plants, animals, and microorganisms are sources of secondary metabolites diversity. Diversity of secondary metabolites requires isolation biodiversity through extraction, fractioation, purification, and structure elucidation of secondary metabolites. Natural products chemistry course examines secondary metabolites contained in an organism [1]. Total of 250.000 species of higher plants grow around the world [2]. About 85.000 species are grown in Latin America, 35.000 species in Africa, and at least 50.000 in Asia [2]. Less than 10% of the world's higher plants has studied the content of secondary metabolites [3]. These natural wealth has a great potential in advancing the natural products chemistry.

Studies of secondary metabolites through laboratory activities in natural product chemistry course useful for third or fourth year undergraduate students who have a basic understanding of the chromatographic and spectroscopic techniques used in the identification of natural compounds. There are at least two important points in natural product chemistry laboratory (i) procedures to isolate secondary metabolites from an organism, and (ii) elucidation structures of secondary metabolites.

PROCEDURES TO ISOLATE SECONDARY METABOLITES FROM AN ORGANISM

Of the hundreds of secondary metabolites that can be isolated from plants, many of them show interesting biological activities such as cytotoxicity [4-6],

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antimalarial activity [7-9], antiviral activity [10-12], antifungal activity [13-15], and antimicrobial activity [16, 17]. These biological activities can be used to guide students to the active pure compound isolation. Various bioactivities show potential for the lead compound to be useful for industrial drug or pesticide industries. Some examples of compounds isolated from plants such as artelastisin (*1*) and artelastin (*2*) isolated from *Artocarpus scortechinii* are shown in Figure 1 [18]. These compounds are flavonoid derivatives.

Isolation of secondary metabolites from various plant species provides an opportunity for students to find evidence to support the concept polar compounds will be soluble in polar solvents and nonpolar compounds will dissolve in nonpolar solvents, or to connect new concepts with the students knowledge to rationalize various phenomena such as the various properties of plants that can be used by humans for treatment. Efficacy of these plants relate to levels of the chemical content in the plants. These activities enhance the meaningful learning in class. Isolation secondary metabolites from plants through the process of extraction, fractionation, purification, and characterization has resulted in many compounds such as artoindonesianin A, B, C [19, 20]. General procedure to isolate secondary metabolites from plants according the following scheme (Figure 2) [21].

Although there is a general procedure for isolating secondary metabolites from an organism, but the same secondary metabolite from a plant species can be isolated in various ways. Futhermore there is no standard procedure to isolate the secondary metabolites of a plant species. These conditions can be used to train the creative thinking skills. The examples of innovative procedures of isolation of pinostrobin from *Kaemferia pandurata* compared with the literature presented in Figure 3 [21]. The active constituent, pinostrobin, is already known in the rhizomes of *K. pandurata* base on the literature [22, 23]. Futhermore students were asked to develop a procedure base on the literature and the results of each stage of isolation process. Procedure of isolation of pinostrobin from *Kaemferia pandurata* were developed by students which were different from the literature. Incompatibility of the results of laboratory implementation with the literature led the students to find their own procedures as seen in Figure 3. Differences in the procedures appear on purification stage and used eluent. Students used recrystallization with n-hexane as a solvent while literature used HPLC [23]. It occurs because the result of fractionation stage showed a crystalin fraction that may be purified through the recrystallization process. These laboratory activities provide opportunities for students to develop their crative thinking skills.

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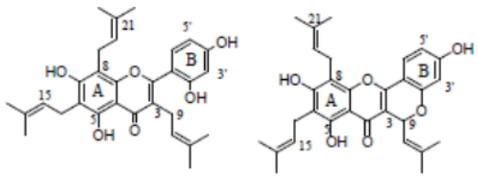

Figure 1. Secondary metabolites from *Artocarpus scortechinii*.

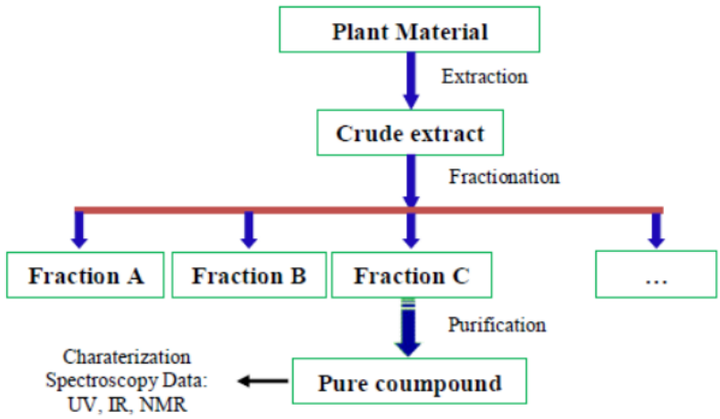

Figure 2. General stages of isolation of secondary metabolites.

ELUCIDATION STRUCTURES OF SECONDARY METABOLITES

Structure elucidation of secondary metabolites based on spectroscopic data. UV (Ultraviolet) spectrum is useful for determining the conjugated double bonds in the secondary metabolites. IR (Infra Red) spectrum is useful for identifying functional groups of secondary metabolites. NMR spectrum (Nuclear Magnetic Reconance) is useful to determine the arrangement of atoms of H and C in secondary metabolites. For examples of structural elucidation of pinostrobin from *K. pandurata* are presented [21]. UV spectra

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were measured with Beckman DU-7000 UV spectrophotometer and IR spectra were recorded on a Perkin Elmer FTIR spectrophotometer. H- and C-NMR spectra were taken on a Agilent 500 MHz spectrometer operating at 500.1 MHz (^{1}H) and 125.8 MHz (^{13}C).

UV spectrum in Figure 4 [λ_{maks} (log ε) 290 (0.99); and 239 (0.302) nm] was consistent with the presence of a flavanone structure.

The IR spectrum in Figure 5 showed absorptions for hydroxyl (3469 cm^{-1}), aliphatic C-H (2972 and 2910 cm^{-1}), and conjugated carbonyl (1643 cm^{-1}).

The ^{1}H-NMR spectrum in Figure 6 included signals for five proton aromatic (B ring) (δppm 7.365, *m*) and signals for two proton aromatic at C-6 and C-8 (δppm 6.050, 2H, *d, J* = 6 Hz). Proton at C-2 can be seen at (δppm) 5.356 (1H, *dd, J* = 13.5; 3 Hz), and signals at at (δppm) 3.072 (1 H, *dd, J* = 13; 4 Hz) and 2.799 (H, *dd, J* = 17; 2 Hz) showed two proton at C-3. Methoxy at C-7 was showed at δ 3.77 (3H, *s*), similar to the arrangement found for a related compound, pinostrobin (*1*). Supporting evidence for the structure assigned to pinostrobin (*1*) came from comparison of the ^{13}C-NMR spectrum in Figure 7. Three carbon Sp^3, twelve proton aromatic, and one carbon carbonil can be found similar to pinostrobin (*1*).

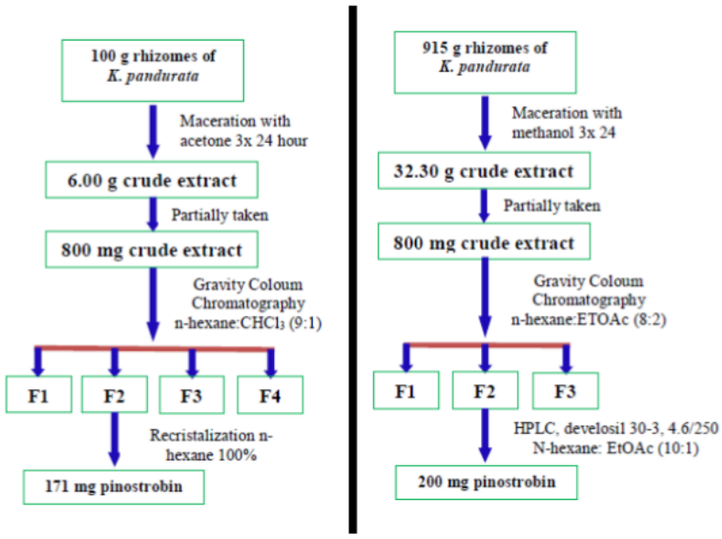

Figure 3. Procedures to isolate pinostrobin from *Kaemferia pandurata*.

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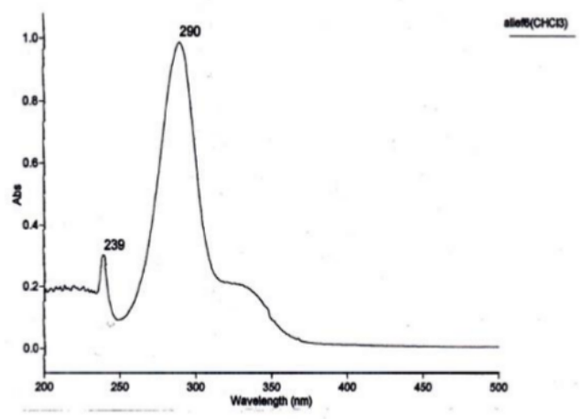

Figure 4. UV spectrum of pinostrobin from *K. pandurata*.

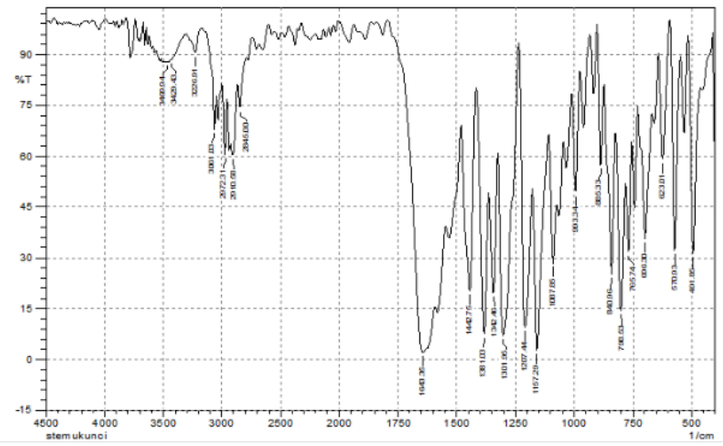

Figure 5. IR spectrum of pinostrobin from *K. pandurata*.

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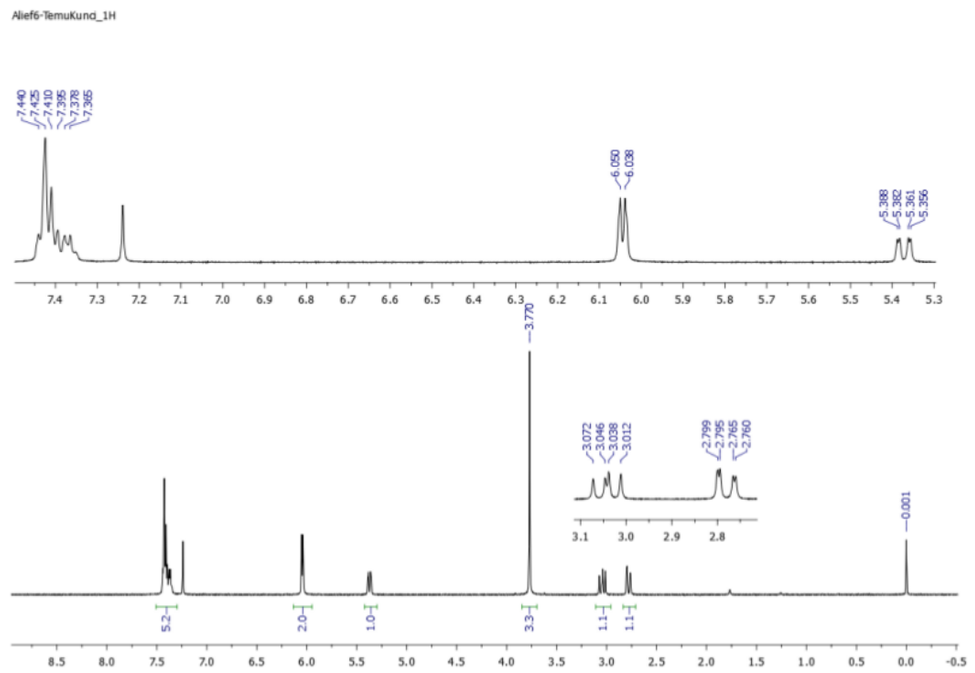

Figure 6. ^{1}H-NMR spectrum of pinostrobin from *K. pandurata*.

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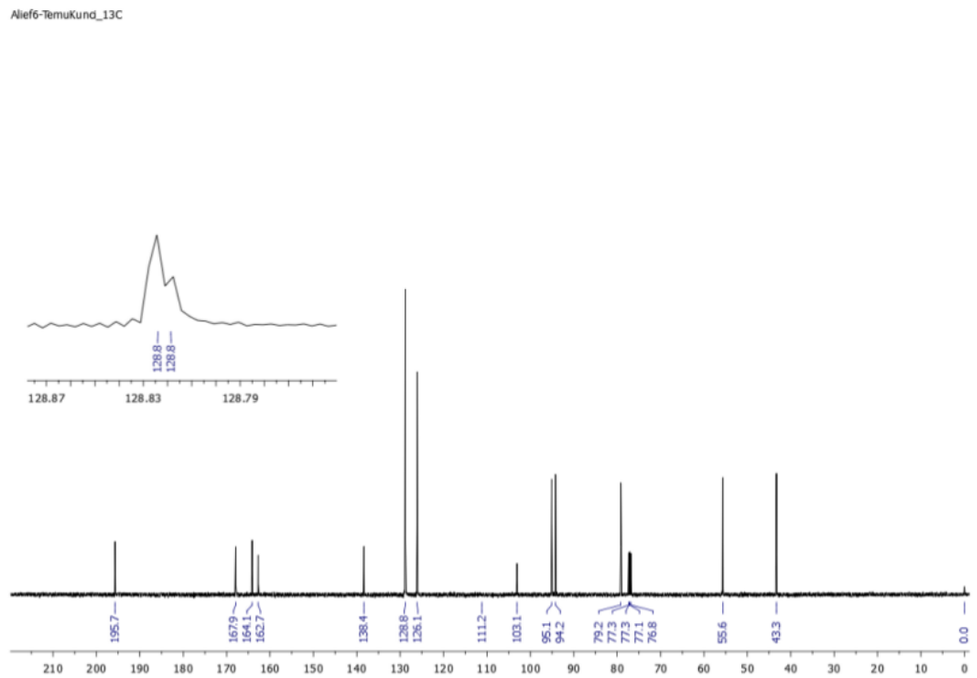

Figure 7. ^{13}C-NMR spectrum of pinostrobin from *K. pandurata*.

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CONCLUSION

One type of laboratory that can be applied in natural product chemistry course is project-based laboratory. Student can be given a project to isolate secondary metabolites from plant species. The sample selection of plant species for natural product laboratory should be considered the level of difficulty of secondary metabolites isolation to avoid any impact on the student frustration due to failure in the laboratory activities.

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