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How does a plant make an antimalarial medicine?

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Abstract

If you think using plants to cure disease is a thing of the past, think again! Today, many medicines in drugstores contain chemicals from medicinal plants. An herb called sweet wormwood (*Artemisia annua*) is one of them. Sweet wormwood is a very effective medicine to treat malaria, the world's deadliest disease. The active ingredient, artemisinin, kills malaria-causing parasites faster than any other medicine. We wanted to understand exactly how this plant makes artemisinin. We knew that sweet wormwood converts another molecule (DHAA) into artemisinin. But no one understood how! Here, we solved this biology problem using chemistry. We tagged DHAA molecules by developing a set of chemical reactions. Using technology, we then monitored the conversion of DHAA to artemisinin. We found this conversion happens spontaneously, without enzymes. Also, it occurs faster in the presence of light. Our understanding of artemisinin formation can help us develop better malaria medicines in laboratories.

Introduction

It's no secret - plants have natural healing powers. Since prehistoric times, people have used *medicinal plants* to treat diseases and improve human health. In fact, most medicines sold in drugstores today contain chemicals from these plants. Often, *biochemists* extract a plant's active ingredient, identify its chemical structure, and then create man-made copies in laboratories. This helps us to lower costs and produce medicine in large amounts, even if there is not enough plant material.

But some other medicines still require the processing of the actual plant material. One of these medicinal plants is *Artemisia annua*, also known as sweet wormwood. Sweet wormwood makes the chemical artemisinin (and its *derivatives*), which is an effective treatment for the world's deadliest disease–malaria.

Malaria is a global health issue. Despite worldwide efforts, about half a million people die from malaria every year. To develop more effective treatments, biochemists have been studying the *antimalarial* properties of artemisinin and how it's made. Malaria is deadly because of the dangerous parasites it creates in the body. We know that the unusual *endoperoxide bridge* in artemisinin kills those parasites. We also know that sweet wormwood makes dihydroartemisinic acid (DHAA) first and then converts it to artemisinin. Previous studies have identified these chemical reactions. But no one understood the last important step - the step where DHAA converts to artemisinin - and how the endoperoxide bridge is formed. We didn't know if it is spontaneous or if it involves enzymes. In this study, we examined this final step.

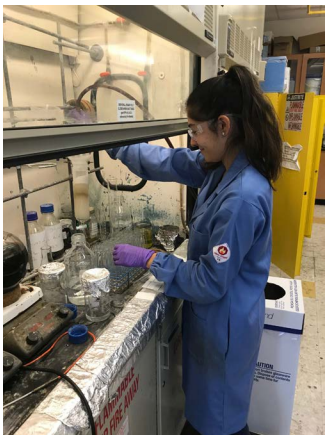
Biochemist Kaitlyn Varela performing experiments with *Artemisia annua*, the plant that produces artemisinin.

Methods

We hypothesized that sweet wormwood converts DHAA to artemisinin spontaneously (without enzymes) and it occurs faster in the presence of light. To test our hypothesis:

1. We created DHAA using previously known chemical reactions and prepared a *solution*. We divided this solution evenly into clear and amber-colored glass containers (40 each). We placed the **clear** glass containers by an open window and we placed the **amber** glass containers in a black box inside a cabinet.
2. Using the method of *nuclear magnetic resonance* (NMR) *spectroscopy*, we watched how the artemisinin formed in the different containers. This helped us identify the chemical structures of the molecules (DHAA and artemisinin) in the containers. But it didn't give us accurate information on how quickly the artemisinin formed, so we revised our method.
3. We developed a process in the lab that replaces two hydrogen atoms in DHAA with *deuterium* atoms. Atoms are made up of protons, neutrons, and electrons. Deuterium has one extra neutron compared to hydrogen and is heavier than hydrogen. Deuterium is called an *isotope* of hydrogen.
4. We used the same experimental setup as before (containers by the window vs. in the cabinet) and this time we analyzed artemisinin formation through *liquid chromatography-mass spectrometry* (LCMS). Using the DHAA with 2 deuterium atoms helped us track how much artemisinin with 2 deuterium atoms formed over time.

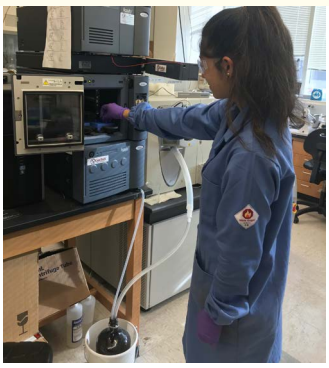
Biochemist Kaitlyn Varela doing research in her lab at the University of Texas in San Antonio



A) Preparing chemical reactions

B) Concentrating material

C) Identifying compounds using NMR



D) Analyzing compounds using LCMS

Results

- We found that the conversion of DHAA into artemisinin is a spontaneous reaction. It does not involve enzymes. The addition of oxygen from the air to the DHAA molecule formed the endoperoxide bridge (Fig. 1).
- Artemisinin formed in all containers. However, the reaction to form artemisinin with 2 deuteriums happened 40 times faster in the clear containers by the window than in the amber containers in the dark cabinet.
- Surprisingly, when the DHAA with 2 deuterium atoms converted to artemisinin, some deuterium atoms were lost.

Please see Figure 1 Page 3

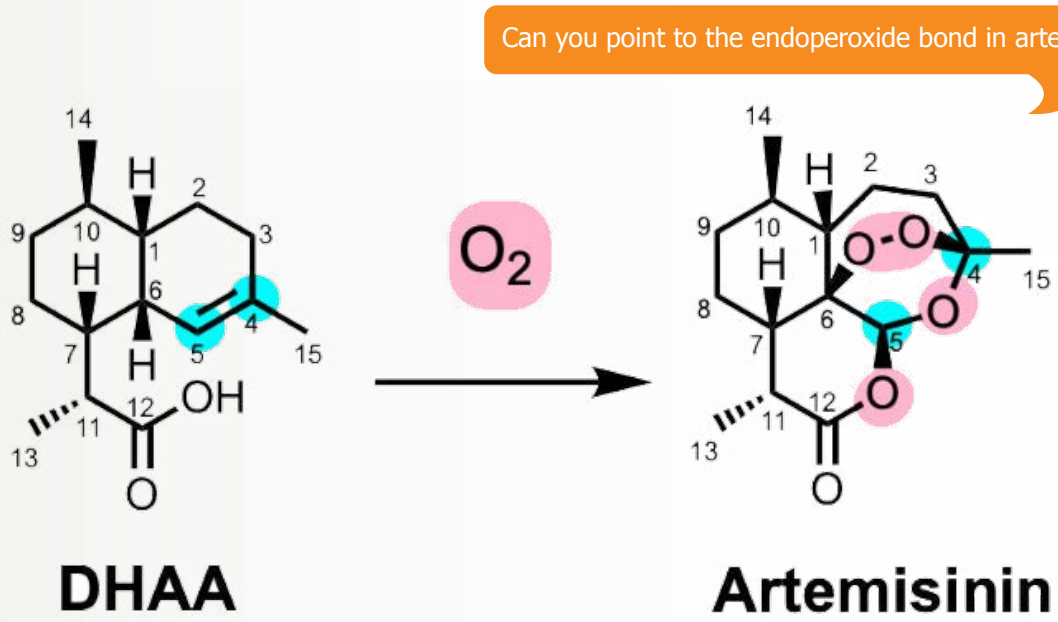


Figure 1:
During the conversion of DHAA to artemisinin, oxygen molecules merge into the molecule to form an endoperoxide bond.

Discussion

We used basic chemistry to discover the details about the biological processes that sweet wormwood uses to create artemisinin. We learned that oxygen atoms move into DHAA and form an unusual endoperoxide bridge during a spontaneous reaction. This structure gives the molecule its ability to attack and kill malaria parasites rapidly.

Despite the efforts for a malaria-free world, wiping it out is a hard task. One reason is that malaria parasites evolve quickly and become resistant to medicines. Furthermore, sweet wormwood availability is low. It takes a long time to cultivate the plant, extract artemisinin, and manufacture the medicine. Understanding artemisinin's antimalarial properties and formation is an important step in our fight with malaria. Our results can lead the way to mass production of effective and inexpensive antimalarial treatments in laboratories. It has the ability to save millions of lives!

Conclusion

Medicinal plants have so much to offer, yet we still have so much to learn. Therefore, the study and conservation of plants is important. Here are some ideas of how you can learn more:

- Research native medicinal plants in your area. Use a book or a phone app (like PlantSnap or Plantifier) to identify them. Bonus: this can help you avoid poisonous plants such as poison ivy.
- Start an herb garden in your backyard or in a container. Begin with a few simple herbs such as chamomile, mint, or rosemary. They will not only look and smell good, but could also help with a sore throat or upset stomach.

Glossary of Key Terms

Antimalarial – a type of antiparasitic (parasite killing) chemical that can be used to treat or to prevent malaria.

Biochemist – a scientist who studies the chemical and physical principles of living things.

Derivatives – a substance that can be made from another substance.

Deuterium – one of the isotopes (see definition below) of hydrogen. It has one proton and one neutron. The most common isotope of hydrogen is protium. It has one proton and no neutrons. Because deuterium contains a neutron, it is bigger and heavier than protium, so it is sometimes called heavy hydrogen. (Like how a person can eat one apple or two apples and would be heavier after eating two apples).

Endoperoxide bridge – a peroxide (-O-O-) group that connects two atoms. This is the unique structure that affects malaria parasites.

Enzyme – a substance made by a living organism that helps make biochemical reactions happen. (Similar to how engines help cars move, enzyme help reactions occur).

Isotope – atoms with the same number of protons and electrons, but a different number of neutrons. (Picture three apples. One has five seeds, another has seven seeds, and the last has twelve seeds. Although they have a different number of seeds, they are all still apples).

Liquid chromatography-mass spectrometry (LCMS) – a chemistry technique to analyze the chemical structure of compounds by showing how much they weigh.

Medicinal plant – plants that act as a medicine for a human or animal.

Malaria – a blood disease transmitted by the bite of infected mosquitoes. Symptoms are chills, fever, and sweating that usually occur a few weeks after being bitten.

Nuclear magnetic resonance (NMR) spectroscopy – analyzing the molecular structure of compounds by observing local magnetic fields around atomic nuclei. (Imagine a class of misbehaving students. The teacher raises his/her voice to get the student's attention [magnetic field is on], the student's behavior improves. After a while, when the teacher is relaxed [magnetic field is off], the students return to their bad behavior. The spectrometer records that process of relaxation).

Solution – a mixture made up of a solute and a solvent. In this case, DHAA (solid) is the solute, and dichloromethane (liquid) is the solvent.

Check your understanding

1. What makes artemisinin an effective antimalarial?
2. Why is it important to understand the structure and formation of artemisinin?
3. At first, scientists couldn't collect accurate data about where artemisinin formed faster: in light or darkness. How did they revise their method to measure formation rates over time?
4. Chemicals from plants (or their man-made copies) are in a variety of medications. Some of these include: taxol to treat cancer, prostratin to treat HIV, and digoxin to treat heart failure. What others can you think of?

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