

Topic 5. Identifying Organic Compounds in Plants

Introduction: This lab will introduce some simple qualitative methods for identifying basic types of organic compounds. These methods will be applied to conduct exercises on photosynthesis and on enzyme action.

I. Carbohydrates

Ia. Simple Sugars

The basic formula for simple sugars is $(\text{CH}_2\text{O})_n$: where “n” is three or some greater number. For some of the most common sugars $n = 6$ and, hence, their formula is $\text{C}_6\text{H}_{12}\text{O}_6$. Sugars with this formula include both glucose and fructose. Both of these sugars react with Benedict’s solution as do all “simple” sugars.

Procedure (work in pairs):

1. Take 5 ml of dilute honey and add 1 ml of Benedict’s solution in a test tube. Heat this tube in beaker of boiling water. What do you observe?

This is a positive test for simple sugars such as glucose and fructose.

2. Repeat this test using a solution of sucrose (table sugar). Do you get a positive reaction?

3. Again add 5 ml of distilled water to a test tube. Place a piece of apple into the tube and crush it with a stirring rod. Pour the water into a clean test tube and test with Benedict’s. Does apple have simple sugars?

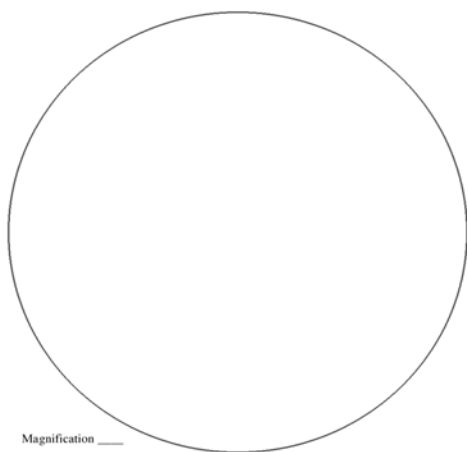
Discussion: The sugars in honey and apple are both monosaccharides. Given that honey is simply nectar gathered from flowers, what is the function of the sugars in nectar and fruit (how do they help the plant to survive to reproduce)?

Table sugar is processed from stems of the grass, sugar cane. Chemically it is the disaccharide, **sucrose**. Each molecule of sucrose consists of one glucose and one fructose bound together. With this chemical bond, electrons are not available to reduce the copper ion in Benedict's solution, hence, the negative reaction. Sucrose is the sugar transported by the phloem of plants.

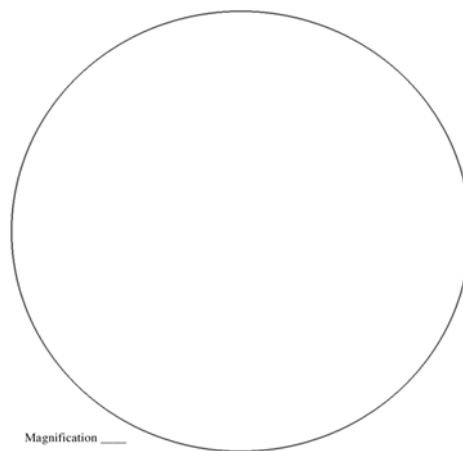
Speculate about why it may be adaptive for plants to produce monosaccharides in fruits and flowers, but transport sugars in their tissues in the form of a disaccharide.

Ib. Starches are long chains of the simple sugar glucose. Starch is easily identified using a solution of iodine and potassium iodide (I_2KI).

1. Fill a test tube one third full of distilled water, and add 2 drops of starch suspension. Swirl the tube and add one drop of I_2KI . This result is diagnostic for the presence of starch.
2. Cut a thin slice of potato and make a wet mount using distilled water. Observe the tissues at 400x and make a drawing. Remove the slide. Put a drop of I_2KI on one edge of the cover slip and blot water from the other edge using tissue paper. Put the slide back under the microscope and observe any changes. Draw the stained tissue.



Unstained



Stained

3. Working with your partner take a precut corn grain and treat the cut surface with I_2KI . Specifically what tissue of the grain tests positive for starch?

- Save this corn section for reference in “Part III”.

4. Testing a Simple Hypothesis using I_2KI (work in pairs).

At the side bench is a variety of *Coleus* with variegated leaves. There are two obvious pigments to be found in various regions of its leaves:

- Chlorophyll in the areas colored green, and
- Anthocyanin in the areas colored red.
- Note that here is also a region of overlap.

Take a leaf from the plant. Note where chlorophyll and anthocyanin are present. Because chlorophyll is not found everywhere in the leaf, we can use the leaf to evaluate whether chlorophyll is necessary for photosynthesis. To do so, however, we require a method for determining photosynthetic activity.

One approach is to consider where in the leaf starch forms. If photosynthesis occurs more rapidly than the photosynthate can be transported out, leaves tend to convert photosynthate into starch. As we have a means for determining the presence of starch, we have everything we need to conduct a simple experiment to evaluate the hypothesis:

Chlorophyll is necessary for photosynthesis.

Procedure:

1. Take a leaf and draw it. Clearly indicate where chlorophyll and anthocyanin are located and where they overlap.
2. Boil the leaf in water to remove the water soluble anthocyanin pigment. Make a second drawing clearly showing where the chlorophyll is located.
3. Boil the leaf in alcohol to remove the chlorophyll pigmentation.
4. Carefully place the bleached and brittle leaf on a watch glass and flood it with I_2KI .
5. Again draw the leaf clearly indicating where the purple stained starch is located.

Drawing 1
Untreated leaf

Drawing 2
Anthocyanin removed

Drawing 3
Stained with I2KI

Discussion:

Are your results consistent with the hypothesis?

Consider a second hypothesis:

Anthocyanin is necessary for photosynthesis.

Do your results support that statement?

Can you reject the second hypothesis?

Have you “proved” the first hypothesis?

II. Lipids

Lipids are not one chemical class of molecules like carbohydrates. However, all lipids are nonpolar: they do not mix in water and they will dissolve certain nonpolar substances that will not dissolve in water. Triglycerides, phospholipids, waxes, and steroids are all examples of lipids. In this exercise we will consider only the triglycerides, which are commonly known as fats and oils.

Procedure: Take a piece of peanut seed, cut a thin slice and make a wet mount of the tissue. Observe the tissue under both low and high power. Now add I₂KI as described previously and observe the distribution of starch in the tissue. Add Sudan IV stain to your wet mount using the same procedure previously described for adding I₂KI to a wet mount. This stain is nonpolar and will move into the lipid droplets residing in the tissue.

Discussion:

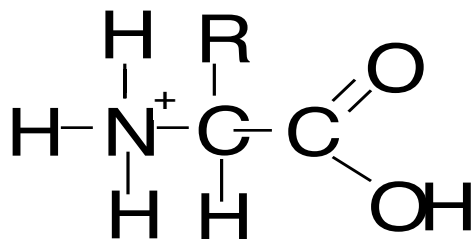
Can you think of why it may be more adaptive for a plant to store food in the form of oils in a seed than in a tuber?

Why don't animals lay down long-term energy stores in the form of starch?

Do animals ever lay down energy stores in the form of starch?

III. Proteins

From your text you know that proteins are polymers of amino acids. The general formula of an amino acid is given below:



There are twenty different amino acids found in living systems. Each of these has a different "R" group. A huge number of proteins can be formed using different combinations of these twenty. One test for proteins uses concentrated nitric acid. The acid reacts with the "R" groups of certain amino acids.

IIIa. Identifying Protein in a Corn Grain (work in pairs).

Procedure: Observe the corn kernel you tested earlier with I₂KI. Note where starch is located. Take another dry corn kernel that has been cut longitudinally, and place it in a petri dish. Add two drops of concentrated nitric acid to the cut surface of the half-kernel: **be careful not to breathe the fumes!!!** Wait three minutes and check for a yellowish coloration indicative of proteins.

Compounds other than proteins will turn yellow after this treatment. To specifically test for the presence of proteins, add two drops of concentrated ammonium hydroxide to the yellowish tissue. Proteins should turn orange after this check step.

WARNING: Both nitric acid and ammonium hydroxide are extremely caustic. Protect your eyes! Use safety glasses while working with the reagents, and avoid rubbing your eyes after using them until after you rinse your hands. Avoid breathing the fumes of either.

Questions and Speculation for Discussion:

In what tissue is starch concentrated?

In which tissue is protein concentrated?

Why are starch and protein located in different regions of a corn kernel?

IIIb. Enzyme Action: An essential role of proteins in living systems is to act as catalysts. A catalyst allows a chemical reaction to occur under a certain set of conditions that otherwise would not. A catalyst is not consumed in the reaction it catalyzes. Proteins that act as catalysts are termed, “enzymes.” Enzymes not only catalyze critical reactions necessary for life, they also insure these reactions produce one primary product which results in efficient use of resources in living systems. One set of enzymes that catalyze the conversion of starch to glucose in plants is diastase. Diastase is a combination of two enzymes, alpha amylase and maltase. These enzymes are produced in germinating grains by a tissue surrounding the endosperm called the aleurone layer. The alpha amylase breaks starch down into the disaccharide, maltose. Maltase breaks the that disaccharide down into glucose.

Procedure: This is to be conducted by each table divided into three teams.

Team 1: Take 8 test tubes and add 8 ml of water and then two drops of I₂KI to each.

Team 2: Take 15 ml of starch suspension from the side bench and add it to a 50 ml beaker. Take a 2 ml sample from this beaker and test for simple sugars using the Benedict’s test following the procedure outlined in *Part A*.

Team 3: This team requires a watch with a second hand. Add 2 ml of diastase solution to the remaining starch suspension in the 50 ml beaker. After mixing the two together take an immediate 1 ml sample and add to a test tube of water-I₂KI. Now take samples at the times listed below and add to each test tube in turn from the left to the right. Categorize your observations of the test tubes by assigning each a number one through five where **5 = purple-opaque** and **1 = completely colorless and transparent**.

0 seconds	_____	1.5 minutes	_____	4 minutes	_____
30 seconds	_____	2 minutes	_____	5 minutes	_____
60 seconds	_____	3 minutes	_____		

Team “2” should then take a 2 ml sample of the starch/diastase mixture and again run a Benidict’s test.

Does it test positive for simple sugars? _____

Discussion:

What was present in the stock solution of starch?

What was present in the beaker after 1 minute?

What was present in the beaker after 5 minutes?

What would be the probable affect on the activity of diastase if the temperature was increased 10°C ?

What would be the probable affect on the activity of diastase if the temperature was increased 50°C ?
