

Topic 4. Identifying Organic Compounds in Plants

Introduction: This lab will introduce some simple qualitative methods for identifying basic types of organic compounds.

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 reducing sugars?

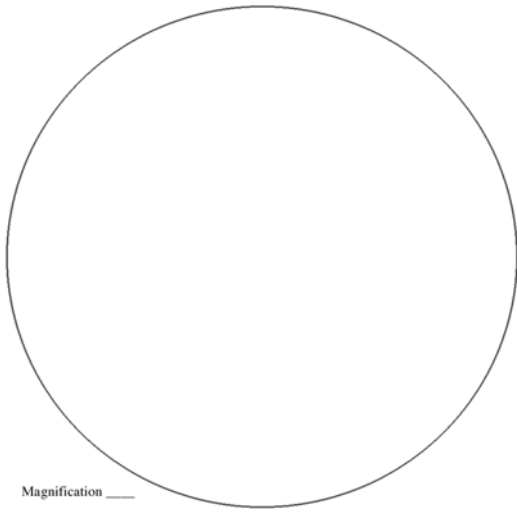
Discussion: The sugars in honey and apple are both monosaccharides. Given that honey is simply nectar gathered from flowers, what is the function of the sugars in nectar and fruit (how do they help the plant to survive to reproduce)?

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.

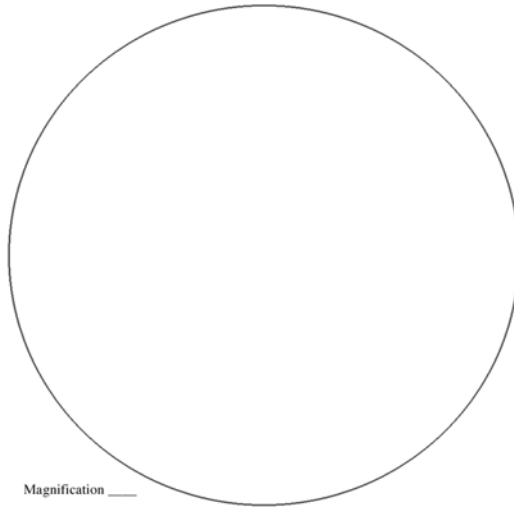
Speculate about why it may be adaptive for plants to produce monosaccharides in fruits and flowers, but transport sugars in their tissues in the form of a disaccharide.

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 soaked 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”.

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 sterols are all examples of lipids. In this activity 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 I_2KI 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 I_2KI to a wet mount. This stain is nonpolar and will move into the lipid droplets residing in the tissue.

Discussion:

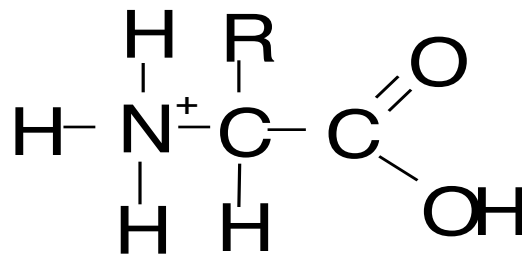
Can you think of why it may be more adaptive for a plant to store food in the form of oils in a seed than in a tuber?

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 a polysaccharide?

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.

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

Procedure: Observe the corn kernel you tested earlier with I_2KI . Note where starch is located. Now take a 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?

IV. Nucleic Acids in Wheat Germ.

Nucleic acids are polymers of nucleotides. Each nucleotide consists of a nitrogenous base, a sugar, and a phosphate group. In the case of DNA, the nucleotides can be guanine, adenine, thymine or cytosine, and, hence, there are four possible nucleotides. The sequence of nucleotides, in some chains of DNA, encodes for the sequence of amino acids in a protein, so, DNA molecules determine the structure and function of plant cells as mediated through the proteins. In section 'III' we discovered that in the grain of corn (a grass fruit), the protein is concentrated in the young plant embryo called the germ. In this activity we will extract the DNA of germ of a different grain, wheat. The DNA encodes for the production of the protein which we saw was concentrated in the embryo.

Procedure: Work in groups of four.

1. Take a test tube and add 1 cm of wheat germ as measured from the bottom.
2. Fill the test tube 40% full with hot water from the coffee urn. Do not overfill!.
3. Cork tightly and shake vigorously for 90 seconds.
4. Add a squirt of dish detergent.
5. Gently rock the test tube back and forth for another 90 seconds.
6. Fill the tube with 95% ethanol.
7. The stringy white precipitate that forms in the alcohol layer is DNA.