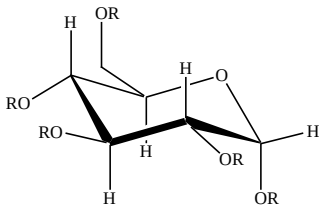
ISOLATION OF CAFFEINE FROM TEA

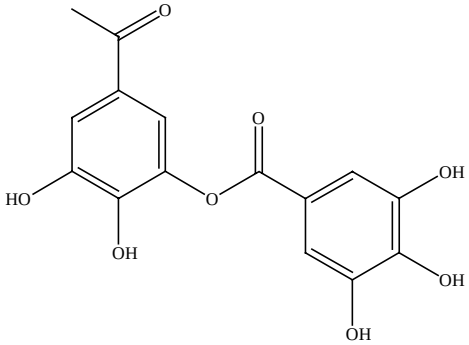
Introduction

In this experiment, caffeine is isolated from tealeaves. The chief problem with the isolation is that caffeine does not exist alone in the tealeaves, but other natural substances from which it must be separated accompany it. The main component of tealeaves is cellulose, which is a polymer of glucose. Since cellulose is virtually insoluble in water, it presents no problems in the isolation procedure. Caffeine, on the other hand, is somewhat water-soluble and is one of the main substances extracted into the solution called tea. Caffeine constitutes as much as 5% by mass of the leaf material in tea plants.

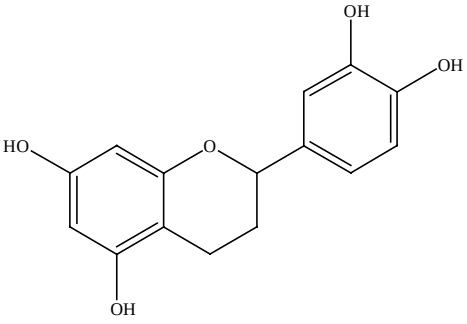
Tannins also dissolve in the hot water used to extract tealeaves. The term tannin does not refer to a single homogenous compound, or even to substances that have similar chemical structure. It refers to a class of compounds that have certain properties in common. Tannins are phenolic compounds having molecular masses between 500 and 3000. They are widely used to "tan" leather. They precipitate alkaloids and proteins from aqueous solutions. Tannins are usually divided into two classes: those that can be hydrolyzed (react with water) and those that cannot. Tannins of the first type that are found in tea generally yield glucose and gallic acid when they are hydrolyzed. These tannins are esters of gallic acid and glucose. They represent structures in which some of the hydroxyl groups in glucose have been esterified by digalloyl groups. The nonhydrolyzable tannins found in tea are condensation polymers of catechin. These polymers are not uniform in structure; catechin molecules are usually linked at ring positions 4 and 8.



Glucose if R = H
A tannin if some R = Digalloyl

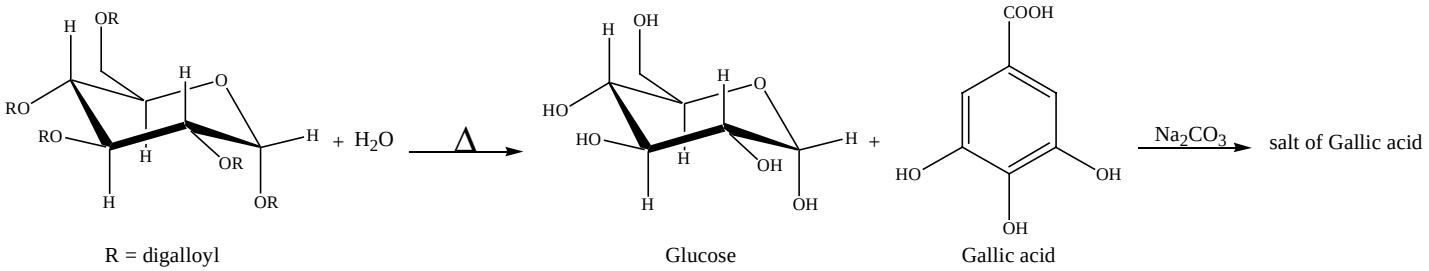


A digalloyl group



Catechin

When tannins are extracted into hot water, some of these compounds are partially hydrolyzed to form free gallic acid. The tannins (because of their phenolic groups) and gallic acid (because its carboxyl groups) are both acidic. If sodium carbonate, a base, is added to tea water, these acids are converted to their sodium salts, which are highly soluble in water.



R = digalloyl Glucose Gallic acid

Although caffeine is soluble in water, it is much more soluble in an organic solvents like methylene chloride or ethyl acetate. Caffeine can be extracted from the basic tea solution with either solvent, but the sodium salts of gallic acid and the tannins remain in the aqueous layer.

The brown color of a tea solution is due to the flavenoid pigments and chlorophylls and to their respective oxidation products. Although chlorophylls are soluble in methylene chloride and ethyl acetate, most other substances in tea are not. Thus, the extraction of the basic tea solution with either solvent removes *nearly* pure caffeine. The organic solvent is than easily removed by evaporation to leave the crude caffeine. The caffeine is then purified by sublimination.

Procedure

This lab will be done in groups.

I. Isolation of Caffeine (week one)

Place 50 mL of water in a 100 mL beaker. Cover the beaker with a watch glass and heat the water on a hot plate on LOW (about 130^{o} C) until it is almost boiling. Get two tea bags. You must measure the mass of tea in the bags. Open your bags of tea carefully, and mass to 0.0001 grams on the balance. (Use the analytical balance in M229 to do this.)

Place the tealeaves into the water. Replace the watch glass and continue heating for about 5 minutes. To separate your tealeaves from the water you may either do a gravity filtration or a suction filtration. Dispose of the tealeaves and filter paper in the appropriate container. Take the filtrate and transfer this liquid to a beaker. Add about 0.50 g of sodium carbonate to the hot liquid and 13 g of sodium chloride. Now, perform suction filtration to remove the solids. For this filtration, coat your filter paper with a small amount of Celite *before* adding the solution to the Büchner funnel. Discard the

solids and filter paper in the appropriate waste container.

Cool the tea solution to room temperature and transfer into a separatory funnel. Obtain about 10 mL of 1-propanol and transfer it to the separatory funnel. The 1-propanol will extract the caffeine. Gently shake the mixture for several seconds and properly vent as you learned previously. Be sure to shake gently to avoid forming an emulsion. In the hood, vent the tube to release the pressure, being careful that the liquid does not squirt out towards anyone. Shake the mixture more vigorously for about 30 seconds, with occasional venting.

Let the separatory funnel rest until layers have formed.

If an emulsion forms, do one of the following to break it up. Otherwise, proceed with the experiment.

1. To separate the layers and break up any emulsion, transfer the contents of the separatory funnel into as many test tubes as needed. Centrifuge the mixture in the test tubes for several minutes; be sure to balance the centrifuge by placing a tube of equal weight on the opposite side. If an emulsion still remains (indicated by a green brown layer between the 1-propanol layer and to top aqueous layer), centrifuge the mixture again.
   Remove the organic layer with a glass pipette and transfer it to a clean container. Be sure to squeeze the bulb before placing the tip of the pipette into the liquid, and try not to transfer any of the dark aqueous solution along with the 1-propanol layer.

2. To separate the layers and break up any emulsion, add some saturated sodium chloride solution to the separatory funnel. Gently stir with a stirring rod to help the emulsion break up.

Remove the organic layer from the separatory funnel and put it into a clean large test tube. Replace the aqueous layer back into the separatory funnel.
Repeat the extraction two more times, just as above, using a fresh portion of 1-propanol each time.

Combine all three organic layers in a large test tube. Remove the aqueous layer from the separatory funnel and transfer the organic layer back into the test tube. Wash the organic layer with 10% NaOH solution. When washing, you are trying to remove *impurities* from the organic layer and therefore you should keep the organic layer, discarding the aqueous layer. Transfer the washed organic layer to a clean, dry large test tube. If there are visible drops of the dark aqueous solution in the test tube, transfer the 1-propanol solution to another test tube using a clean, dry glass pipette. If necessary, leave a small amount of the 1-propanol solution behind in order to avoid transferring any of the aqueous mixture. Dispose the aqueous waste in the appropriate container.

Add a small amount of granular anhydrous sodium sulfate to dry the organic layer. If all the sodium sulfate clumps together when the mixture is stirred with a spatula, add some additional drying agent. Remember, we want the drying agent to be loose and "look like a snow globe." Allow the mixture to stand for 5-10 minutes. Stir occasionally with a spatula.

Decant or transfer the dried 1-propanol solution with a glass pipette into a dry 100 mL round bottom flask, leaving the drying agent behind.

Evaporate the 1-propanol using the rotary evaporator (rotovap). When the solvent is *almost* evaporated, remove the flask immediately. The last milliliter or so of 1-propanol will easily evaporate from the heat of the flask. The crude caffeine should coat the bottom of the flask. Do NOT continue rotovapping after the solvent has evaporated, or you may sublime some of the caffeine (and thus decrease your yield). After the flask has cooled and the rest of the 1-propanol is evaporated, rinse the residue with two successive 5 mL portions of acetone. Combine the acetone and place it in a large, labeled and massed (to 0.0001 g) vial. Do NOT cap the vial. Cover the opening with a piece of filter paper and secure it with a rubber band. Place the vial in the large beaker provided for this purpose on the side counter and let the acetone evaporate over the week.

II. Purification of Caffeine (week two):

Before purification, mass the vial with your crude caffeine (to 0.0001 g) from last week's lab. Scrape out as much caffeine as possible into a large test tube. *Do not clean the vial*. You will use the tiny bits of crude caffeine you are unable to transfer for a TLC plate later.

Caffeine can be purified by sublimation. Assemble a sublimation apparatus (to be demonstrated – please see your instructor for further instructions before proceeding). The cold-finger condenser is already inserted into a cork. Make sure the cold-finger is *clean* and ***dry***. Use tubing to connect the water inlet and outlet (in the bottom, out the top); make sure your hoses fit snuggly so that water doesn't leak out.

Insert the cold-finger into the large test tube that your crude caffeine has been placed in. There should be about 2 cm between the caffeine and the cold-finger. Heat the sample gently and carefully with a micro burner to sublime the caffeine. Hold the burner in your hand (hold it at its base, not by the hot barrel) and apply heat by moving the flame back and forth under the flask and up the sides. If the sample begins to melt, remove the flame for a few seconds before you resume heating. When sublimation is complete, discontinue heating. Allow the apparatus to cool.

When the apparatus is at room temperature, carefully remove the cold finger with the cork still attached. If this operation is done carelessly, the sublimed crystals may be dislodged from the tube and fall back into the flask. Scrape the sublimed caffeine onto a tared piece of smooth paper and determine the mass of caffeine recovered to 0.0001

grams.

Determine the melting point of your purified caffeine product. Describe your product.

Now, prepare and run a TLC plate with three samples: your crude caffeine, your purified caffeine, and the reference caffeine provided. Use ethyl acetate to dissolve the caffeine samples (remember – you want a 1% solution, so you will need to use *very* little of each solid) and as the developing solvent. Be sure to properly label your plate! You will have to look at the plate under UV light to see the spots. Once done marking spots, encase your plate with the clear tape provided. Dispose of all waste in the appropriate containers.