Extraction and isolation of Caffeine from tea leaves

Reading assignment: ***Techniques in Organic Chemistry*** 2nd ed pages 158-159. 3rd ed pages 198-199.

General background and overview of the experiment:

Humankind has historically used advantageous compounds derived from plants and animals. For instance, various extracts from plants have been used as teas, potions, medicines and poisons. Though these extracts can contain a mixture of many different chemicals, often only one or few are responsible for the activity of the extract. For decades such extracts were used directly from their natural sources. This practice has many disadvantages, as the composition of the extract can vary from time to time and it is highly dependent on the availability of the natural source. At the beginning of the 19th century chemists made the first attempts to isolate the active components within these natural mixtures. The first compound to be isolated and purified was morphine from opium. Sertürner accomplished this by extracting opium with hot water and precipitating morphine with ammonia. He obtained colorless crystals that were poorly soluble in water but soluble in acids and alcohol. To make sure that the effect of the compound was identical to that of raw opium he tested the crystals on himself…

Following this initial experimentation, many natural products were isolated and their structures were determined. Once a structure was elucidated, chemists were able to devise synthetic methods to synthesize these compounds. This was the beginning of modern pharmaceutical chemistry. Today, researches are still looking for new compounds from natural sources. Potential drugs are often isolated from sea creatures, like sponges or slugs, parts of plants that were used in traditional medicine, or new a species discovered in the rain forest.

In this lab we will extract and purify caffeine from tea leaves. First water soluble compounds will be extracted from dry tea leaves with boiling water. Then, caffeine will be preferentially extracted from the water into organic solvent. The solvent will be removed and the crude material will be purified by sublimation.

Caffeine extraction

Caffeine (Figure 1) belongs to a group of compounds known as alkaloids. Alkaloids are a diverse group of compounds that are found primarily in plants and contain basic nitrogen atom(s). The basic nature of these compounds makes them exists mostly as salts. Other well-known alkaloids include morphine, strychnine, quinine, ephedrine, and nicotine.

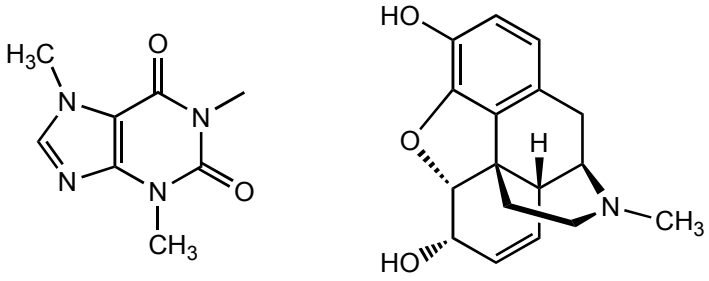

Figure 1. Structure of caffeine (left) and morphine

The major sources for caffeine are the seeds of the coffee plant (*Coffea Arabica*), cola nuts, Mate which is used as tea in Paraguay and Tea leaves (*Camellia Sinensis*). Three types of tea are commercially produced from tea leaves (green, oolong and black) which differ only in their processing methods. To obtain green tea, fresh leaves are steamed to destroy the natural enzymes that cause fermentation. If however, the leaves are allowed to ferment an enzymatic oxidation process occurs which gives rise to oolong tea. Longer fermentation times yields black tea.

Choosing extraction conditions for isolation of a product from its natural source depends on both the properties of the compound and the composition of the source. In our case caffeine is soluble in both water and organic solvents. It is possible to extract caffeine from leaves by solid/liquid extraction to hot water. The medium polarity of caffeine implies that it can be further separated from water soluble compounds by extraction to a polar non-protic solvent like methylene chloride ($\boldsymbol{K}_{CH_2Cl_2/H_2O} \sim 10$).
Designing an efficient extraction scheme requires analyzing the major components of tea leaves. These include cellulose, proteins and amino acids, tannins, pigments and saponins.

Cellulose
The major component of leaves is cellulose, which serves as a rigid and insoluble structural element. Cellulose is a linear polymer composed of D-glucopyranose units connected through carbons 1 and 4. The fully equatorial conformation of β-linked glucopyranose residues stabilize the chair structure of the ring and minimize its flexibility. Although cellulose carries many hydroxyl groups, it is not water soluble due to its high molecular weight.

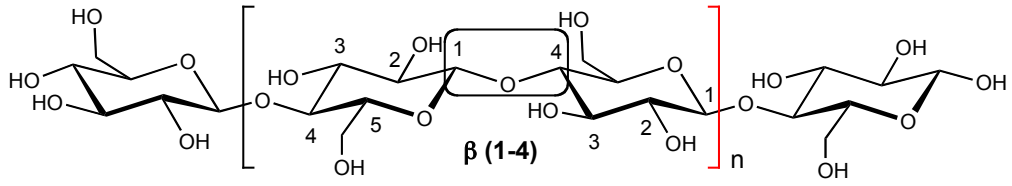

Figure 2. Structure of cellulose.

Tannins
Tannins are polyphenolic compounds (having OH on aromatic ring…) with molecular weights of 50-20,000. Tea tannins are soluble in water and therefore extracted from the leaf and responsible for the typical bitter taste of tea. Tea tannins belong to a subgroup named hydrolysable tannins. The core structure is D-glucose, to which several units of gallic acid are attached, via ester bonds. (Figure 3) It is important to note the gallic acid is poly functional, and able to form multiple ester bonds.

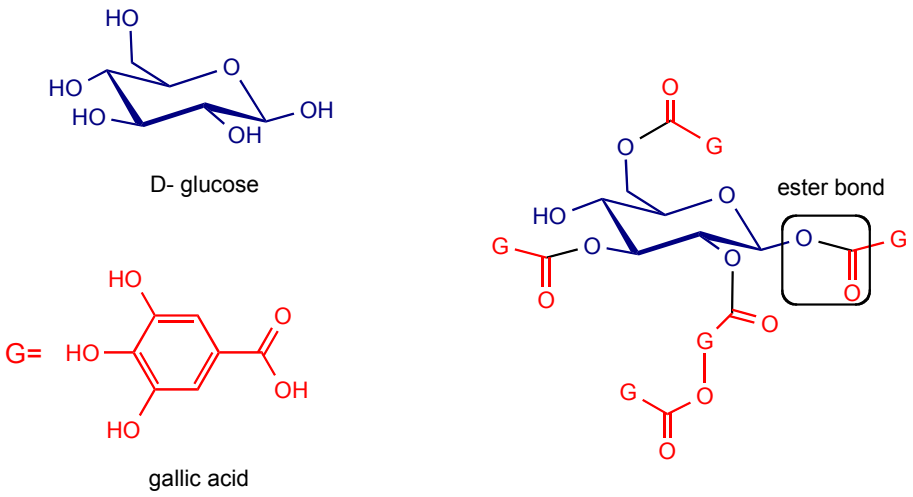

Figure 3. Typical structure of hydrolysable tannins. Note that multiple units of gallic acid are attached by ester bonds.

The presence of soluble tannins in tea leaves complicates the isolation of caffeine, as low molecular weight tannins are also soluble in methylene chloride. However, we can take advantage of the chemical reactivity of the ester bonds in hydrolyzble tannins. When tea leaves are boiled in the presence of weak bases, such as $CaCO_3$, the ester bond is cleaved. This cleavage produces glucose and a calcium salt of gallic acid. These very polar compounds will stay in water and will not be extracted into the methylene chloride. Additionally, the base also converts caffeine molecules the may be present as salts to the free base, increasing its solubility in methylene chloride.

Proteins and pigments

Proteins and pigments are very soluble in water and therefore do not present problems to the separation of caffeine by extraction. The content of pigments varies between different kind of teas and the level of oxidation that the leaves were exposed to.

Saponins

Saponins are compounds that have amphiphilic structure, i.e. molecules having a polar water-soluble group attached to a water-insoluble hydrocarbon moiety. This amphiphilic nature gives saponins detergent-like properties, which increases the solubility of organic molecules in water. Saponins may therefore induce the formation of emulsions during extraction, complicating the separation. Emulsions can be broken by addition of salt to the aqueous layer (therefore increasing the polarity of the aqueous phase and reducing the solubility of organic molecules in it) or by centrifugation.

Overview of the extraction process

The following figure shows a schematic representation of the extraction process that will give "crude" caffeine after evaporation.

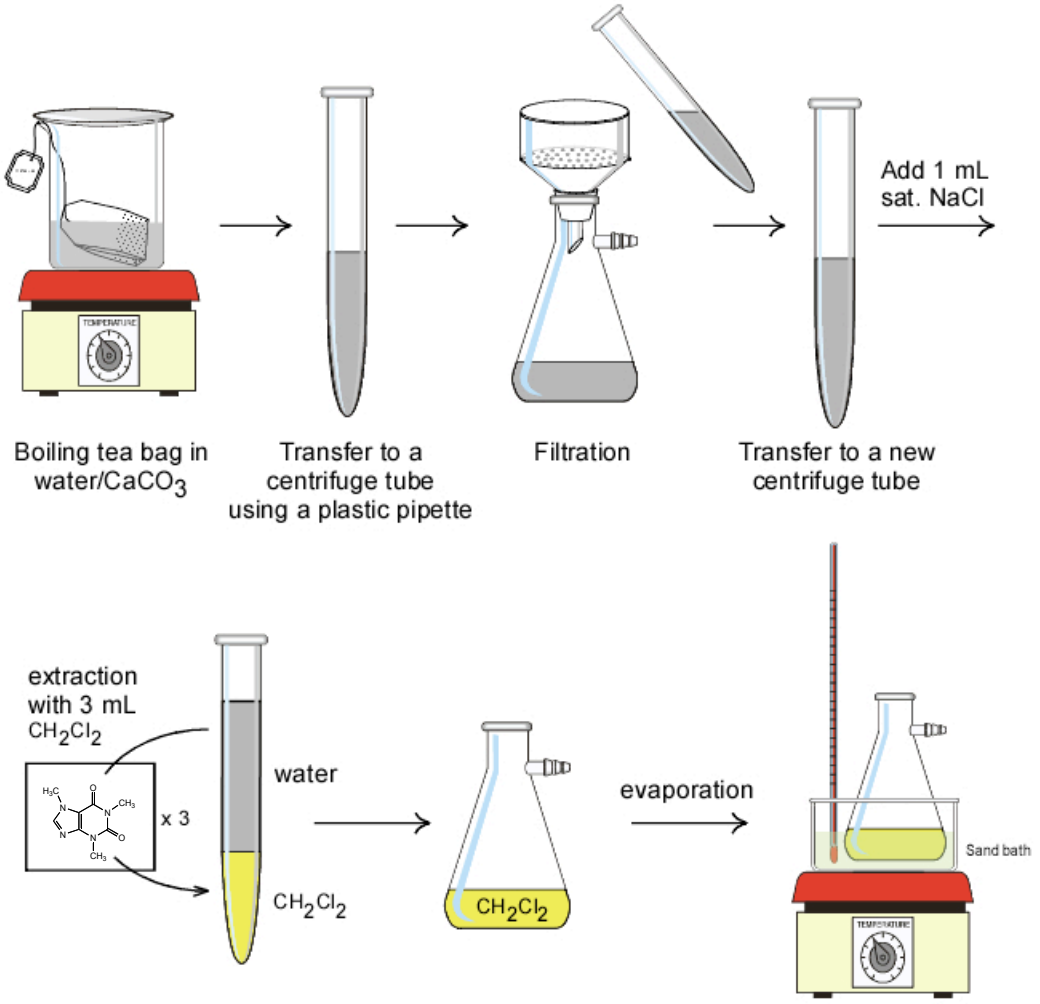

Figure 4. Schematic representation of caffeine extraction

Purification by sublimation

After extraction and evaporation the product that is collected still contains considerable amounts of impurities. One method of purification is based on the ability of caffeine to sublime. Sublimation is the ability to pass directly from solid state to the vapor state and condense back to a solid form without passing through liquid phase (figure 5). Since the impurities in crude caffeine extract do not sublime under the same condition as caffeine, sublimation will result in pure caffeine. The sublimation method will be described in the procedure.

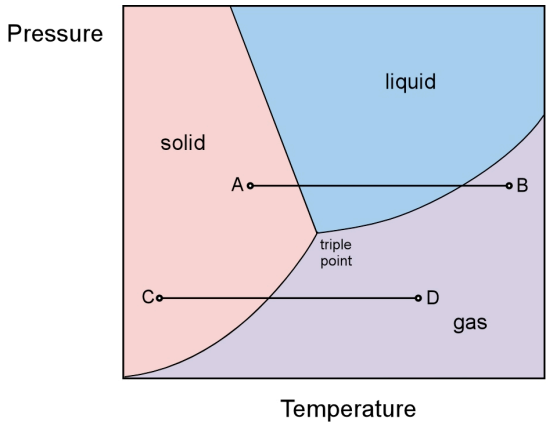

Figure 5. Phase diagram of hypothetical compound. Transition from A to B describes melting of solid to liquid followed by evaporation to gas. Transition from C to D describes sublimation from solid to gas.

Some biology for your general knowledge: How and why caffeine effect us?

The consumption of caffeine results in wakefulness and alertness. The activity of caffeine results from its structural similarity to adenosine (figure 6) and cyclic adenosine monophosphate (cAMP). This structural resemblance allows caffeine binding to the active site of receptors or enzymes that normally reacts with adenosine derivatives.

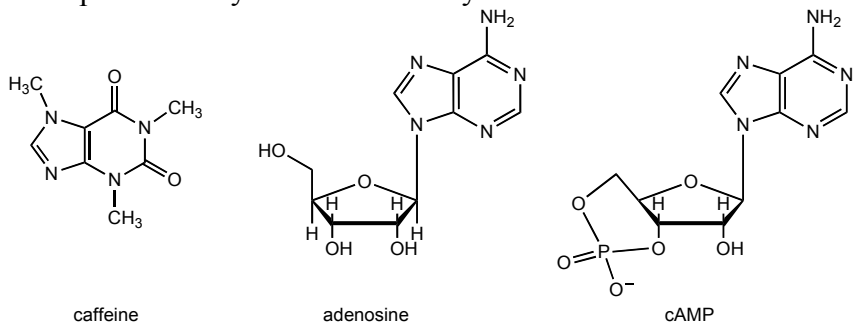

Figure 6. Structural similarities between caffeine and adenosine derivatives.

Adenosine has an important role in regulation of brain activity. During the day there is a buildup of adenosine levels in the brain and when the concentration is high enough, adenosine binds to brain receptors that in turn activate mechanisms leading to drowsiness and sleep. When caffeine binds to the adenosine receptors it prevents binding of adenosine and therefore delays sleepiness. Caffeine also inhibits the enzyme cyclic nucleotide phosphodiesterase (cAMP-PDE) that converts cAMP to noncyclic monophosphate. cAMP is a secondary messenger that activates processes that lead to increasing blood pressure and delivering more oxygen to the brain. Normally these effects of cAMP are reversed by its enzymatic hydrolysis by cAMP-PDE. When caffeine binds to cAMP-PDE it stops the breakdown of cAMP, and therefore its excitation effects are prolonged.

Procedure

Isolation of caffeine from tea leaves

Soilid-liquid Extraction: Obtain one tea bag from your TA and record its weight. Note that the average weight of empty bag is~0.2g. Place the bag inside a 100 ml beaker (leaving the label outside). Add ~0.8 g of calcium carbonate and 15 ml of water. Add a boiling chip, cover the top of the beaker with a 70 mm watch glass and bring the solution to a gentle boil on a hot plate. Boil for 20 minutes while stirring it gently from time to time with a glass rod. Allow the solution to cool to room temperature and then remove the bag from the solution. Gently squeeze the bag on the sides of the beaker with glass rod to allow removal of maximum solution from the bag. Be careful not to puncture the bag while doing this.

Removing the solids: Let the solids sink to the bottom of the beaker. Using a plastic pipette, transfer the liquid into a centrifuge tube. Centrifuge down the solids and then remove the liquid into a Buchner funnel equipped with #1 filter paper (use 50 ml flask and remember that you always turn on the vacuum before adding the solution). **Be careful not to transfer the solids into the funnel as this will dramatically slow the filtration**. Wash the solid that are left in the centrifuge tube twice with 0.5 ml DI water and then transfer the sediment into the funnel and allow the residual solution to filter.

Extraction into organic solvent: Using a plastic pipette transfer the solution from the filter flask into a centrifuge tube. Add one ml of saturated sodium chloride. Extract the solution with 3 ml methylene chloride (don't forget to release the pressure by removing the cap after shaking). If an emulsion is formed, it can be broken by centrifuging the solution for 2 minutes. Transfer the organic layer into a clean centrifuge tube. (In some cases a third layer is observed between the organic and aqueous phases. In this case transfer only the bottom clear methylene chloride layer. Repeat the extraction two more times with fresh portions of 3 ml methylene chloride and combine the organic layers. Wash the combined organic fractions with 1 ml saturated sodium chloride solution and separate the organic layer. Dry the organic layer by adding anhydrous sodium sulfate. Remove the dry organic layer into a pre weighed 50 ml filter flask. Wash the drying agent twice with 1 ml methylene chloride and add it to the filter flask. Evaporate the methylene chloride using a sand bath (in the hood !). Make sure that the temperature of the sand is not higher then 80^{o}C! Weigh the dry flask to find the "crude" product weight. Keep a sample for melting point.

Caution! Methylene chloride is toxic and a possible carcinogen. Minimize exposure to its vapors by working in the fume hood. Use double gloves when handling methylene chloride.

Purification by sublimation

First we need to convert your filter flask into a sublimator. Place a filter adaptor on top of the flask and insert inside it a centrifuge tube (see figure 7). Place the flask inside a sand bath (don't forget to secure it with clamp) and connect the side arm to the vacuum line (don't forget the "water trap"). Gently turn on the vacuum and fill the inner centrifuge

tube (also known as "cold finger") with crushed ice. Start heating the assembly (~200 ^{o}C) and observe how the caffeine sublimes and collects on the cold finger. When no more deposition of product is observed, remove the flask from the sand bath. Carefully take out the cold finger and scrape off the product onto a weighing paper. Some of the product will be collected on the sides of the filter flask. Scrape it off the flask and collect it. If the two fractions that you collected are looking different than don't combine them and analyze each one separately. Weigh the product obtained and measure it's melting point. Calculate the percentage of caffeine that was isolated from the crude and the percentage of caffeine in dry tea leaves. Submit the product to your TA with the lab report.

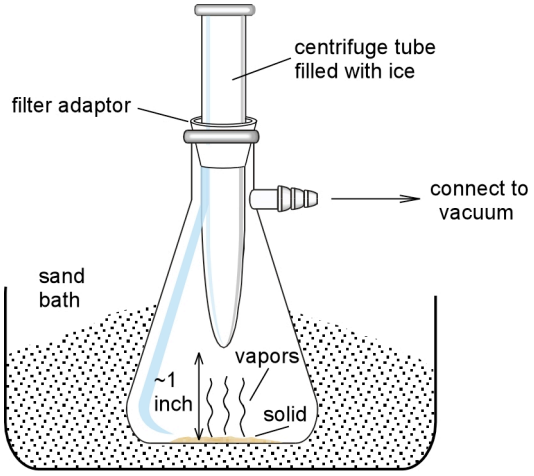

Figure 7. Assembly of sublimator

Prelab questions:

1. Write an equation describing the hydrolysis of one ester group in tannins by $CaCO_3$.
2. How would longer roasting time of green coffee beans effect its caffeine content? Why?