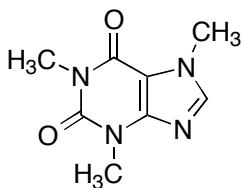
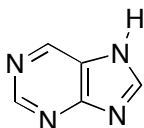


Organic Chemistry 211 Laboratory

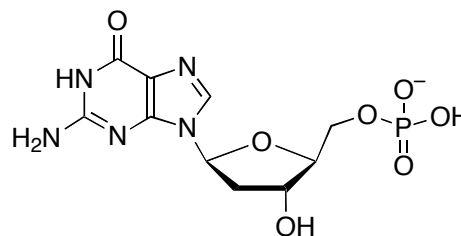
Isolation and Characterization of a Natural Product: Caffeine.



caffeine



purine



2'-deoxyguanosine
5'-phosphate

In this experiment, the techniques of solid-liquid extraction and liquid-liquid extraction will be used to isolate caffeine from tealeaves. *A new purification technique, sublimation, will be used to perform the final purification.*

Caffeine is a member of the class of natural products called alkaloids, which contain nitrogen. Other members of this class include morphine, mescaline, and strychnine. The strong biological activity of these compounds has led to their thorough investigation by many different disciplines. Caffeine possesses the oxidized skeleton of purine, a relative of which can be found as a constituent of DNA (see above). Caffeine is found in many plants with coffee and tea being the most familiar. A cup of coffee or tea provides a 25- to 100-mg “Dose” of caffeine, which stimulates the central nervous system in a widely exploited manner.

From the proceeding, it is not surprising that caffeine is soluble in hot water, but it is also soluble in dichloromethane, allowing the following extractive protocol to isolate it. This two-step extractive isolation assures that ionic species remain in the aqueous layer while taking advantage of the swelling that water causes in the tea leaves. Simply extracting the tealeaves with dichloromethane directly is ineffective. The sodium carbonate added to the water during the “steeping” stage is to insure that the caffeine and organic acids are deprotonated. Thus, caffeine will be free to dissolve in dichloromethane while the carboxylate salts will not (see Experiment 3). The caffeine supplied by extraction is fairly pure, but sublimation will further purify the material in a procedure that is effective with the small amounts obtained here.

Procedure

With care, measure out 1-2 grams of tealeaves. To a 50-mL Erlenmeyer flask, mass 1.1 g of anhydrous sodium carbonate, and then add 10 mL of deionized water. Heat this mixture on the hot plate/stirrer with swirling to dissolve the sodium carbonate. Add the tea to this solution, in the Erlenmeyer flask. Cover the mouth of the Erlenmeyer flask with a watch glass and heat the mixture to a gentle boil.

After 30 minutes of heating, cool the Erlenmeyer flask to room temperature and transfer the aqueous extracts (through a simple gravity filter) to a separatory funnel. To assure that all the aqueous extracts are transferred, rinse the filter paper and the tealeaves in it with about 2 mL of hot water (You should preheat some water). The tealeaves may be disposed

of, at this point. Add 2.0 mL of dichloromethane to the extracts and swirl to mix. A bad emulsion may form, but this can be broken up using a stirring rod. Otherwise, transfer the emulsion to a 12-mL centrifuge tube (make sure that it is glass not plastic), and then briefly centrifuge. A clear and colorless dichloromethane layer will have separated from the dark aqueous layer. Carefully remove the lower dichloromethane layer using a long disposable pipette. Repeat the dichloromethane extraction process 4 more times, and collect the organic layers in a *tared* small Erlenmeyer flask. Dry the wet dichloromethane extracts with anhydrous sodium sulfate and collect the dried solutions in a 25-mL Erlenmeyer flask, using a pipette filter (a Pasteur pipette plugged with a cotton plug). After passing all the dried solution, take a fresh 2 mL portion of methylene chloride, and rinse the remaining sodium sulfate in the original Erlenmeyer flask to assure the complete transfer of all the caffeine.

In a fume hood, add a *tared* boiling stone or magnetic stir bar to the combined dichloromethane extracts and rinsing and heat on the hot plate/stirrer until dry to give an off-white crystalline solid (the crude caffeine). Measure the mass of the crude caffeine you just obtained.

The crude caffeine is further purified by sublimation using an apparatus constructed from a flared test tube fitted with an O-ring and a heavy walled test tube with side arm. The sublimation apparatus pieces will be available in the lab. Transfer the crude caffeine to the heavy walled test tube. Close with the flared test tube and attach to an aspirator with an intervening vented water trap.

Apply vacuum to the apparatus for 1 minute to remove any volatiles before filling the flared test tube with a small amount of crushed ice. Gently heat the heavy walled test tube with a burner taking care to move the flame continuously and not to melt the solids. If melting does occur, remove the flame for several seconds until it solidifies again. Continue heating carefully. Discontinue heating when no more caffeine is observed to sublime. Allow the apparatus to cool under vacuum for 1 minute, open vent on trap to release the vacuum, and then carefully detach the sublimation apparatus to avoid knocking the sublimed caffeine from the chilled condenser. Scrape the caffeine from the condenser with a spatula onto weighing paper.

Measure the mass of the purified caffeine. Determine the melting point of the pure caffeine and compare to the literature value. Since caffeine easily sublimes, you should seal the capillary tube used for the melting point determination (the melting point of caffeine is above its sublimation point). This can be accomplished by charging a melting point capillary as normal and then flaming the open end shut using a burner. The sealing should be performed in a separate room, due to the hazard associated with open flames in the organic chemistry laboratory.

For the report:

- Determine the mass of the crude caffeine in 1 gram of tealeaves.
- Determine the %Recovery for the sublimation process.
- Determine the melting point of the pure caffeine.
- Obtain the IR spectrum of the pure caffeine, analyze it in your report, and also compare it to the IR spectrum obtained from literature sources.
- Predict the $^1\text{H-NMR}$ spectrum for caffeine.

Needed (per student):

Tealeaves (ca. 1-2 grams)-----→ 15-30 grams per lab
sodium carbonate (ca. 1.1 grams)-----→ 16.5 grams per lab
 CH_2Cl_2 (ca. 20 mL)-----→ 300 mL per lab
 Na_2SO_4 -----
Crushed Ice (small amount: to fill a test tube for sublimation)-----