EXTRACTION AND PURIFICATION OF NATURAL PRODUCTS FROM NUTMEG

Natural products are substances that are isolated from living organisms. Parts of the plant or animal that contain the desired natural product are dried and homogenized prior to its isolation. Some methods for the isolation of natural products include distillation, steam distillation, hot or cold expression or extraction with a suitable solvent. The product is usually an oil that consists of a mixture of compounds.

Natural products can be divided into two groups: primary metabolites and secondary metabolites. The metabolic pathways for the biosynthesis of carbohydrates, proteins, fats and nucleic acids are the same in all organisms. These processes demonstrate the fundamental unity of all living matter, and are a part of primary metabolism. The compounds involved in the primary metabolism pathways are called primary metabolites. Unlike primary metabolites, secondary metabolites are restricted to specific organisms and the function of most of them is not yet known. Some secondary metabolites (e.g. toxins and antifeedants) provide defense against predators, some are used for communication among organisms of the same or of different species, and others are coloring agents, hormones, etc. There is no sharp distinction between primary and secondary metabolites and there is considerable overlap between the two groups.

Steam Distillation

Steam distillation is a method for the isolation of compounds that have a relatively high vapor pressure and are immiscible with water. Steam distillation is based on the fact that the total vapor pressure of the mixture of immiscible liquids is the sum of the vapor pressures of its individual components:

$$P_{total} = P_A + P_B + P_C + \ldots$$

A liquid boils when its vapor pressure equals atmospheric pressure. Hence, a mixture of immiscible liquids will have a lower boiling point than the boiling point of its most volatile component (i.e. the one with the highest vapor pressure or the lowest boiling point). That is a consequence of the fact that the vapor pressure of a mixture of immiscible liquids must be higher then the vapor pressure of its most volatile component. Only compounds with relatively high volatility (the compounds that have a high vapor pressure, or in other words low boiling points) distill with steam. Non-volatile compounds do not distill with steam. Therefore, steam distillation is a method for separation of volatile compounds from non-volatile ones. While the volatile components will distill with steam, the non-volatile ones will remain with the residue.

In the past, steam distillation was a common method for isolation and purification of organic compounds. Nowadays, it is mainly used to isolate essential oils from plants. These oils are mixtures of compound with various degrees of complexity that have relatively low boiling points. On the basis of their chemical composition, constituents of essential oils can be divided into two groups: those that contain principally aromatic compounds, produced by the shikimate biosynthetic pathway, and those that contain predominantly aliphatic compounds (terpenes), produced by the mevalonate biosynthetic pathway. Terpenes belong to a larger class of natural products known as lipids. Although many oils contain both groups of compounds (aromatics and terpenes), one usually predominates.

Solvent Extraction

Solvent extraction is an important method for the isolation of natural products. While in steam distillation volatile compounds are isolated, in a solvent extraction compounds soluble in

the particular solvent, or mixture of solvents, are isolated. Since most organic compounds we wish to isolate are insoluble in water, a variety of organic solvents such as hexane, petroleum ether (a mixture of hydrocarbons with low boiling point), diethyl ether, dichloromethane (methylene chloride), ethanol and acetone are among the solvents used for extracting natural products. Carbon tetrachloride is one of the best solvents used in the extraction of essential oils. However, because it is damaging to the ozone layer its use has been prohibited by the Montreal Protocol. Recently, use of ìsupercriticalî carbon dioxide as an extraction solvent (actually either supercritical or sub-critical is used in the extraction process) has gained importance. After extraction, an individual compound must be isolated from the oil and purified by such techniques as distillation, extraction, chromatography or crystallization.

Natural Products form Nutmeg

Nutmeg, the seed of an East Indian tree (Myristica fragrans, Myristicaceae), is the source of natural products in this experiment. Various lipids can make up as much as 35 - 40% of the dried weight of a nutmeg seed. It is important as a spice and its oil is used in flavoring, aromatherapy and perfumery. While the main components of the oil of nutmeg are terpenoids (sabinene, α□ □and β□ □pinene and terpinen- 4-ol), most of the flavor (actually fragrance) comes from the minor aromatic constituents - myristicin and elemicin (Figure 1). Myristicin, also found in parsley and carrots, is a colorless oil that is toxic if ingested in large amounts.

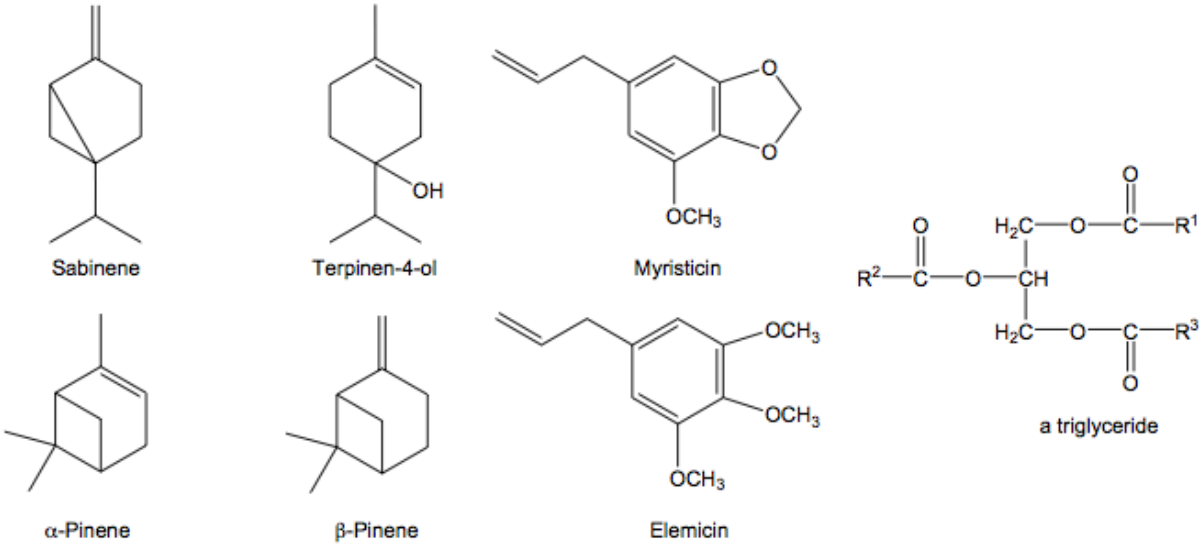


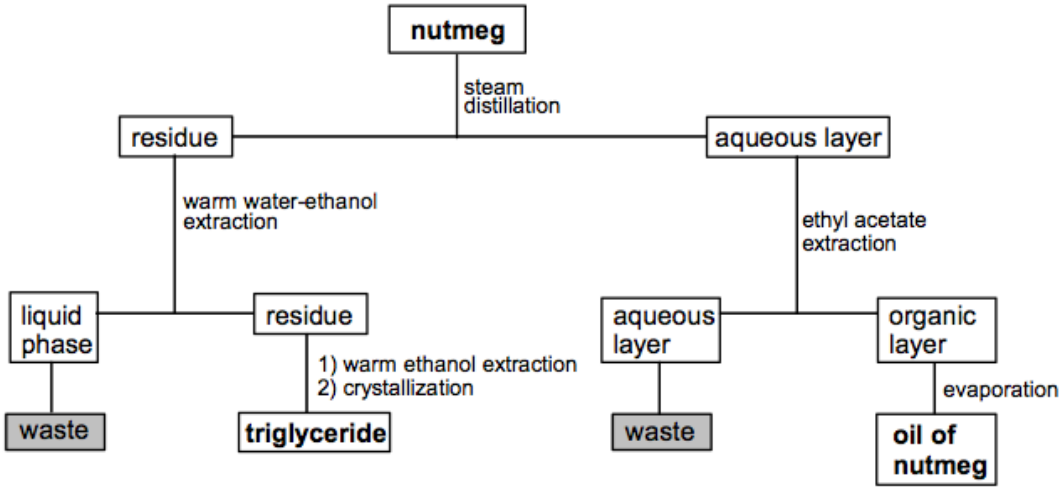
Figure 1. Some of the lipids and aromatics present in nutmeg.

The seeds of plants are frequently rich in a class of lipids known as triglycerides (fats and oils) (Figure 1). Triglycerides are triesters of glycerol and straight-chain carboxylic acids called fatty acids (Table 1). The only difference between a fat and an oil is that a fat is a solid while an oil is a liquid at room temperature. Many different triglycerides exist, since there are many naturally occurring fatty acids available to combine with glycerol's three hydroxyl groups. If all three acids are the same the ester is called a simple triglyceride. If two or more fatty acids are different the ester is called a mixed triglyceride. Most fats and oils are composed of a mixture of different triglycerides (usually mixed triglycerides). Nutmeg is remarkable in that the triglyceride in it is mainly a single simple triglyceride.

Table 1. Some of the Common Fatty Acids

Name	Formula
butyric	$CH_3(CH_2)_2CO_2H$
caproic	$CH_3(CH_2)_4CO_2H$
caprylic	$CH_3(CH_2)_6CO_2H$
capric	$CH_3(CH_2)_8CO_2H$
lauric	$CH_3(CH_2)_{10}CO_2H$
myristic	$CH_3(CH_2)_{12}CO_2H$
palmitic	$CH_3(CH_2)_{14}CO_2H$
stearic	$CH_3(CH_2)_{16}CO_2H$
oleic	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2H$
arachidonic	$CH_3(CH_2)_4(CH{=}CHCH_2)_4CH{=}CH(CH_2)_2CO_2H$

In this experiment you will do a steam distillation of ground nutmeg followed by a solvent extraction (warm ethanol will be the solvent) of the residue. See Scheme 1 for a block-scheme for the isolation of each of the natural products from nutmeg. After the steam distillation is completed, you will add a small amount of ethanol to the residue in the distillation flask. The residue consists of the remaining ground nutmeg and some remaining water (it will look like a thick paste). The added ethanol will make a mixture with the remaining water. The ethanol-water mixture is not suitable for a solvent extraction of the desired natural product. However, it will dissolve colored impurities as well as any remaining oil of nutmeg. You should separate this ethanol-water extract and keep it for TLC analysis. On the remaining purified nutmeg residue you should do another extraction with warm ethanol (this is the actual solvent extraction) and collect the natural product.



Scheme 1. Flow chart for the isolation of natural products from nutmeg.

EXPERIMENTAL PROCEDURE

CAUTION: Ethyl acetate and ethanol are all highly volatile and flammable solvents. Use no flames in the laboratory during this experiment.

1. Place 2 g of ground nutmeg, 35 mL of water and boiling chips in a 100 mL round bottom flask.
2. Assemble a distillation apparatus (Figure 2). On the distillation adapter place a stopper in place of a thermometer and have a separatory funnel (125 mL) in place of a receiving flask.

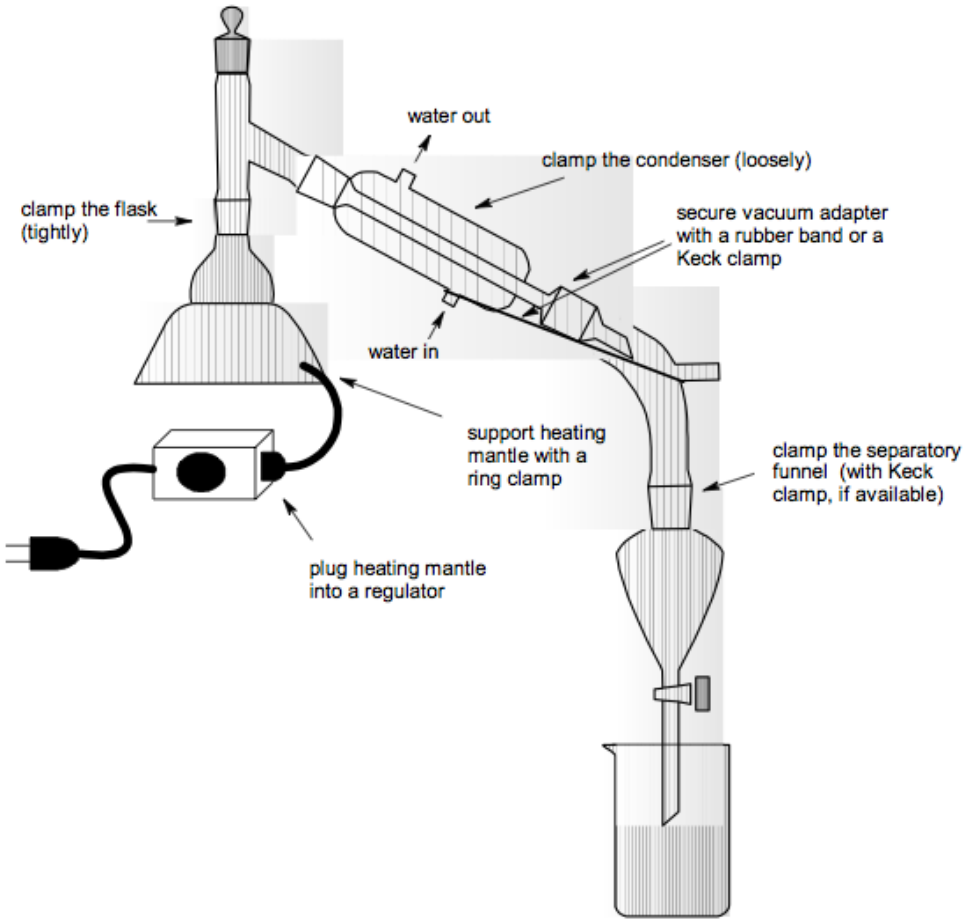


Figure 2. Steam Distillation Apparatus.

3. Place 10 mL ethyl acetate in the separatory funnel. Mark the level of the ethyl acetate in the separatory funnel.
4. Place a beaker, or, if there is enough space, a graduated cylinder under the separatory funnel.
5. Wrap the distillation flask with aluminum foil, turn the heating on high and allow the distilled water to collect in the separatory funnel.
6. After approximately 10 mL of water has distilled (when the water level reaches the 10 mL mark in the separatory funnel), drain the bottom (aqueous) layer and measure its volume. Replace the stopper on the distillation adapter with a glass funnel and return the water to the distillation flask. Do not forget to replace the funnel with the stopper after you finish returning the water.
7. Keep track of the amount of distilled water. You should distill a total of 80 mL of water. You may be wondering how to distill 80 mL of water when you placed only 35 mL into the distillation flask. After you distill the first 10 mL of water, drain it from the separatory funnel and return it to the distillation flask. You should return the first five 10 mL portions (for a total of 50 mL) of the distilled water to the distillation flask. You should distill three additional 10 mL portions of water (for a total of 30 mL). **Do not return those final 30 mL of water to the distillation flask.** Monitor the distillation closely. **You must not distill to dryness!** Nutmeg will begin to burn and the extraction will be ruined. When distilling the final 10 mL of water, remove the aluminum foil (caution: foil may be hot!) so that you can see the contents of the distillation flask. If you notice any smoke in the distillation flask, stop the heating immediately. If you stop the heating in time, you can still save your experiment.

8. After the distillation has been completed only a small amount of water will remain in the distillation flask (5 mL or less). Disassemble the distillation apparatus and allow most of the remaining water to evaporate from the warm distillation flask.
9. Pour the ethyl acetate extract from the separatory funnel into a 25 mL Erlenmeyer flask and add a small amount of anhydrous magnesium sulfate to it.
10. After 10 minutes filter the ethyl acetate extract through a fluted filter paper into a pre-weighed flask. Give the labeled flask to your instructor. Analysis of this solution will be next week once the solvent has evaporated. Next week, the final weight will be recorded to be able to calculate the mass yield of the isolated oil and content of the essential oil (oil of nutmeg) in nutmeg (as a mass %). You will also take an IR spectrum of the oil.
11. After the **distillation flask** has cooled down, add 10 mL of ethanol, place the condenser on the flask and heat the suspension to reflux for 5 minutes (Figure 3).

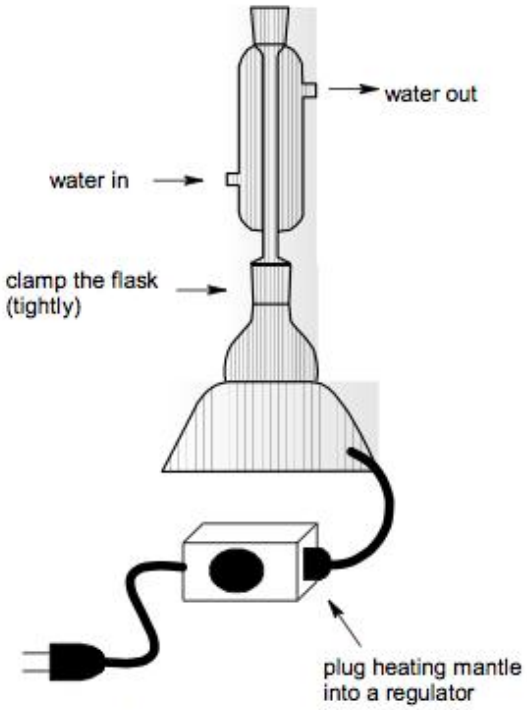


Figure 3. Reflux Apparatus.

12. Gravity filter the warm ethanol extract through a funnel into an Erlenmeyer flask or a beaker. Use a small piece of cotton instead of filter paper in the funnel. Try to decant the liquid portion only and not pour any pieces of nutmeg into the filtering funnel.
    This is your Extract 1.
13. If any pieces of the nutmeg get onto the filtering funnel, return them to the distillation flask. You can return them along with the cotton. The cotton will not affect the extraction. Add 20 mL of fresh ethanol and heat the mixture to reflux for 15 minutes.
14. Filter the warm ethanol solution through the funnel plugged with cotton into a clean beaker or Erlenmeyer flask. This is your Extract 2.
15. Allow both extracts (Extract 1 and Extract 2) to cool and then place them in ice baths.
16. Use a Buchner funnel to vacuum filter the solid. Filter each extract separately. The solid isolated from Extract 2 is the pure triglyceride. Any solid obtained from Extract 1 is crude (impure) triglyceride.

17. Calculate the mass yield of the triglyceride and content of triglyceride in nutmeg (as mass %). Store the solids when you are done.
18. Determine the melting point of Extract 1 and 2. [NOTE: Before taking the melting point, the recrystallized trimyristin should dry exposed to the air for at least overnight or Week 2]. Save any solid obtained from Extract 1 for TLC analysis. If no solid has been isolated, save the liquid Extract 1 for TLC analysis.
19. Perform TLC analysis on the crude triglyceride, the recrystallized triglyceride, the isolated acid, and the oil. The developer is hexanes/ether (3:1 by volume). Place the plate under UV light and observe; while under the light circle the spots with a pencil.
20. Perform IR analysis on Extract 2 and the oil of nutmeg. Identify the major functional groups. Campare your results to the know spectrum provided.
21. Save all final products. These products will be used next semester.

REPORT Suggestions:

Introduction- describe the objectives of the experiment and the methods you are going to use to accomplish them.

Experimental - make references to experimental procedures. Report any changes made to the referenced procedures.

Results- make sure that you only include observations in this section (do not discuss the results here).

Discussion - discuss the results. Discuss purity, yield, melting points and other data that helped you identify the fatty acid and the original triglyceride. Be careful about your claims. Make sure that you have evidence for them. The Questions and Exercises section should help you write the discussion.

- Classify each of the isolated products as either a primary or a secondary metabolite.
- Which of the isolated materials is responsible for the characteristic fragrance of nutmeg?
- How do you know you actually isolated a new product rather than just recovered the starting material? Give at least two different methods to show this.
- Look up the melting points of the fatty acids in the Merck Index or the CRC Handbook of Physics and Chemistry. What is the identity of the isolated acid? Give its trivial and IUPAC names.
- What is the structure of the original triglyceride extracted from nutmeg?
- According to the TLC analysis, how many components accompanied the crude triglyceride?
- What information could you obtain from the IR spectra of the isolated products?

Conclusion - briefly summarize your findings and relate the outcomes to the objectives of the experiment. If possible, make suggestions for future work.