Experiment 9: EXTRACTION AND HYDROLYSIS TRIMYRISTIN PROM NUTMEG SEED

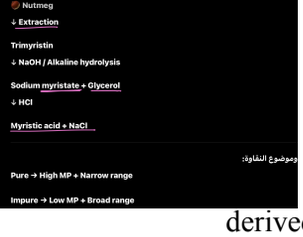
OBJECTIVE: To extract triglycerides (fatty acid) from nutmeg and saponification reaction fatty acids, soap.

This experiment will probably require two laboratory periods.

In general, isolating organic compounds from these living organisms is a difficult task because there are usually numerous compounds in a living organism that makes isolating a particular compound difficult. However, in the case of nutmeg, this isolation is eased when extracting the compound trimyristin because it is found in substantial quantities, often exceeding 25% of the weight of nutmeg but usually below 40% (Weldegirma, 2018). The compound Trimyristin is a triester containing a glycerol backbone with chains of carboxylic acids from the myristic acid. In the case of Trimyristin, the composition is atypical because the three long chains of carboxylic acids are identical. In order to isolate the compound Trimyristin in nutmeg, it is necessary to conduct a procedure referred to as extraction. This extraction procedure consists of removing the solid aspect of nutmeg to a liquid specie, which will be the solvent in the extraction procedure. Then, after the isolation of Trimyristin is completed, it will be crucial to recrystallize to ensure the purity of the compound. This is done by dissolving the adulterated product in a heated solvent which is then allowed to cool and crystallize. Finally, in order to test the purity of the product, the melting point apparatus is employed. Subsequently, it is also crucial to mention the hydrolysis of esters. This hydrolysis of esters, usually referred to as acid and base catalyzed hydrolysis, yields the anion of a carboxylic acid and an alcohol. This is typically caused by the addition of a strong base to a fatty bond, which leads to the production of fatty acid crystals because the triglyceride ester bonds are hydrolyzed (Weldegirma, 2018). In the case of trimyristin, the hydrolysis that takes place is alkaline hydrolysis, which turns into the sodium salt of the myristic acid (Weldegirma, 2018). Moreover, testing for the purity of the compound via the melting point provides information of whether the compound is mixed, and thus impure. To illustrate, the mixed melting point concept states that a pure sample will melt at a higher and broader temperature than an impure sample, which usually melts at a temperature that is lower and broader than the literature melting point value. Due to this, even an equal mixture of two compounds that have the same melting point will result in a lower melting point overall because it is an adulterated mixture.

Subsequently, the reaction that takes place to convert trimyristin to myristic acid is illustrated below (Figure 3), this procedure illustrates the hydrolysis process that takes place when hydroxides are added to trimyristin allowing for the procedure to continue. Then, hydrochloric acid is utilized to yield the desired product, myristic acid, and sodium chloride. However, there is also a potential side reaction that could take place. This side reaction is referred to as the elimination reaction, and could be avoided by keeping the reaction at a low temperature and not overheating the product during the distillation procedure.

Commercially, saponification of triglycerides derived from natural products yields a mixture of fatty acids. Fatty acids derived from plants are usually richer in unsaturated fatty acids than those



Glycerol

↙ ↓ ↘

Myristic — Myristic — Myristic

derived from animals. The triglyceride derived from nutmeg is unusual as it contains only one fatty acid. The fatty acid obtained in this case is saturated. Since saturated fatty acids are solids and often can be distinguished using melting range measurements, nutmeg is a very convenient starting material for this experiment. On the negative side, saturated fatty acids contribute to health problems. The salts that are obtained as a result of saponification before the acidification step were discovered more than 5000 years ago and are still used today as soaps.

Myristic acid acts as a surfactant, opacifying agent, texture enhancer, emollient, cleansing agent and emulsifier in cosmetics and other personal products. There are many salts and esters derived from myristic acid that are also used in personal care products.

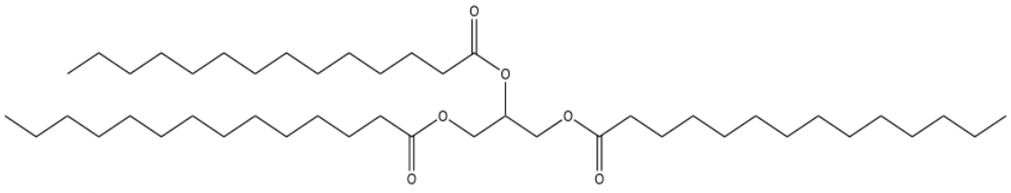


Figure 1. Structure of trimyristin, $C_{45}H_{86}O_6$

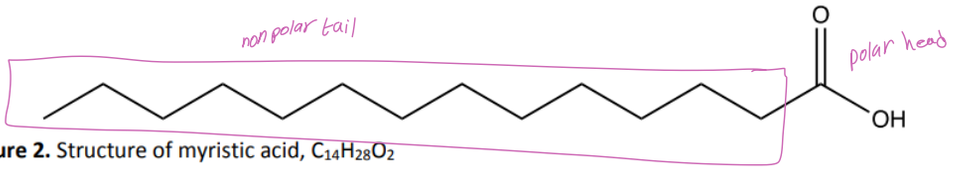


Figure 2. Structure of myristic acid, $C_{14}H_{28}O_2$

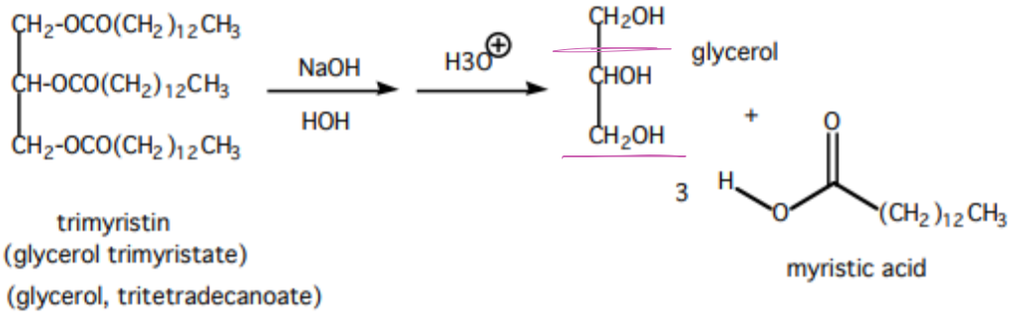


Figure 3. Conversion of trimyristin to myristic acid

Apparatus and materials: two method of extraction, one is used.

Procedure : first method of extraction

1)Weigh 10 g of grounded seeds of nutmeg , transfer it to thimble.
2)Add the thimble inside the soxhlet.
3)Extract for 30 hours with 200 ml methylene chloride (in water bath) and put the condenser above the soxhlet
4)Concentrate the extract in a rotary evaporator
5)Add 100 ml ethanol to the still warm crude fluid trimyristin (that is the red sticky substance remaining) in the flask and heat on water bath to dissolve the crude trimyristin.
6)Let it cool in the refrigerator by continuously stirring with a glass rod (to avoid the separation of oil on the surface)
7)Filter the crystals through the Buchner funnel, and wash with ice-cold ethanol.
8)Dry the crystals in a desiccator.
**Apparatus :
Soxhlet 100 ml
Round bottom flask 250 ml
Evaporating (porcelain) dish 20-25 cm
Vacuum flask 250 ml
Porcelain dish 8 cm
Erlenmeyer flask 250 ml
** Solvents: Ethanol 300 ml, methylene chloride 200 ml
1.Round bottom flasks (100 ml) 2. Erlenmeyer flasks (100 ml) 3. glass peads 4. Hot plate 6. Quickfit thermometer 7. Filter funnel 8. Condenser 9. Filter paper
Chemicals: Nutmeg Powder, n-hexane; sodium hydroxide

PROCEDURE: second method of extraction

PART I: Extraction of myristic acid

1. Place 10 g of nutmeg powder into 100 ml round bottom flask.
2. Add 40.0 mL of n-hexane and 5 boiling stones. Then set up for reflux (heat) the mixture for 30 minutes.
3. allowed the mixture to cool at room temperature then set up for simple filtration (filter the n-hex 100mL Er. Flask).
4. Added few ml of n-hexane into 100 mL R. bottom flask (weight empty RB.Flask), set up for simple filtration, (Note distilled until remain 10mL in R. bottom flask).
5. Calculate t percentage of trimyristin in nutmeg powder. Get the myristic acid melting point.

lf the sample is impure boiling methanol and recrystallize by dissolving the solid in a small amount of then adding water- dropwise (while maintaining boiling) until there is the first hint of cloudiness. Cool and collect the recrystallized product using vaccum filtration.

Part 2 (saponification):

6. The same R. B. flask added (0.2 g, NaOH, 20 ml ethanol), then set up for reflux for 20 minutes.
7. Allow the mixture to cool to room temperature and add 15 mL of ice-water to the mixture. Pour the reaction mixture slowly with vigorous stirring into a beaker containing one mL of 1 mL con. HCI
8. After precipitation is complete, filter Wash the collected solid on the filter with ice-cold water.
9. After the sample has dried, determine the yield and the melting range of the solid.

PART 3: Thin-layer chromatography

10. Prepare a TLC plate (3x7 cm dimension) : Handle it only on the edges, as fingerprints contain UV-active materials. Using a pencil draw a very light line on the sheet (short dimension) about 1 cm from one end. Then make 3 small marks at even intervals along the line for spotting the samples. Draw alight line about 0.5 cm from another end of the plate for the solvent front.

11. Obtain a TLC chamber and place a solvent, dichloromethane to 0.5 cm height. Place a piece of filter paper around the inside surface container and extend into the solvent. A glass jar with a lid or a beaker a watch glass or a cover of a Petri dish can be used as a TLC chamber

12. Use clean capillary tubes, careful. The spots should be as small as possible, to minimize tailing and overlapping when the TLC plate is developed.

13. After the spots are dry Place the TLC plate in the developing chamber. Then gently close the champer. When the spot has moved to the front line, remove the plate. Lay it on a clean surface in fume hood or well ventilated area.

15. Visualize the plate under UV ligh. Immediately draw a light pencil line around each spot. Measure all the distances traveled by the compounds and solvent. Calculate the retention factor (Rf) for each compound.