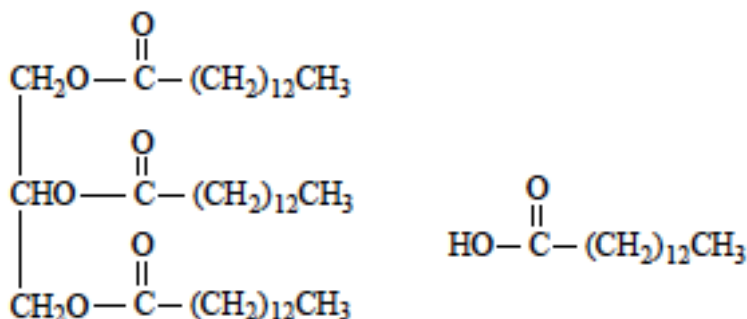


## 12BL Experiment 2: Extraction & Saponification of Trimyristin from Nutmeg

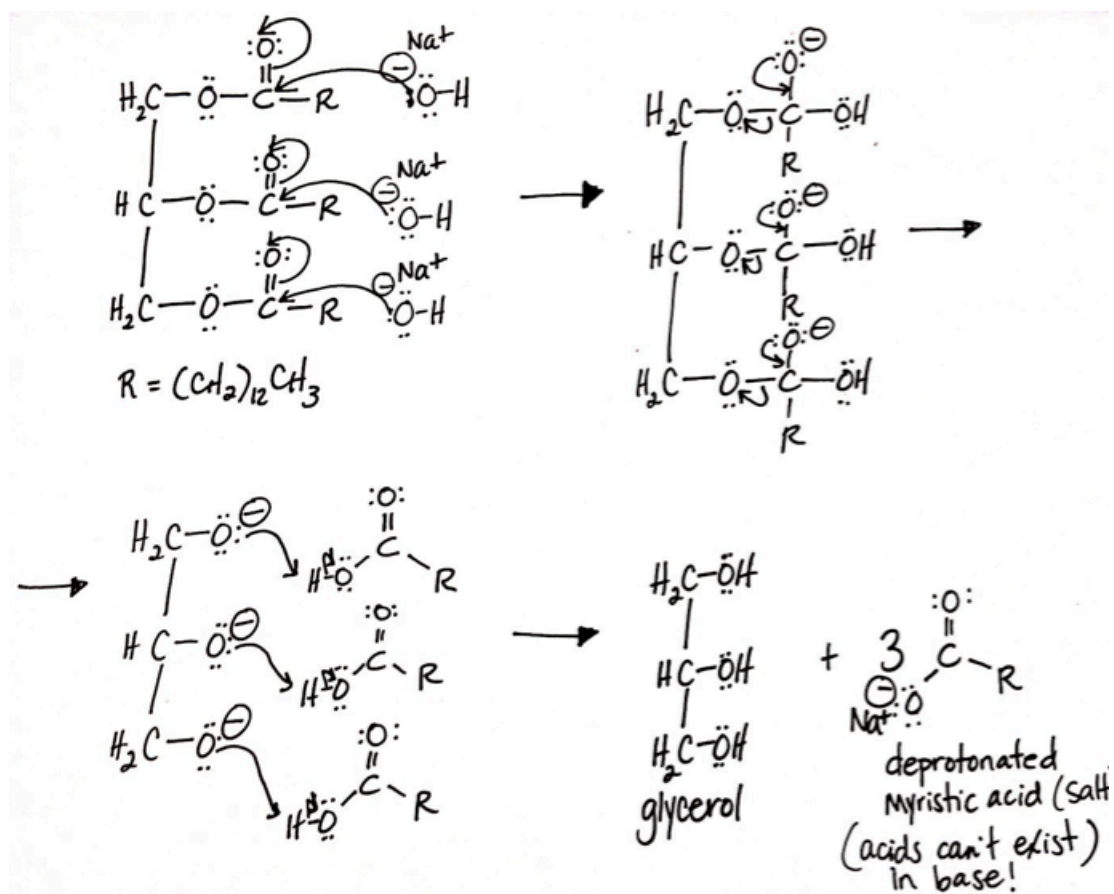
**Safety:** Proper lab goggles/glasses must be worn (even over prescription glasses). Heating of organic solvents releases irritating and sometimes toxic vapors, and often times, volatile solvents splatter vigorously out of their containers! As always, ask where organic waste containers are located in the lab.

**Background:** Trimyristin, a tri-ester or triglyceride, is found in high abundance in nutmeg (*Myristica fragrans*), so its isolation is relatively straight-forward. Esters are organic molecules containing the RCOOR functional group and commonly impart the characteristic fragrance of plants. Trimyristin can be hydrolyzed or saponified to its corresponding fatty acid, myristic acid. Myristic acid is an organic compound containing the carboxylic acid functional group RCOOH. Myristic acid is used in the food industry as a flavoring agent. Myristic acid is found widely distributed in fats throughout the plant and animal kingdom. Many Americans consume very little myristic acid because this fatty acid is found in coconut oil and dairy fats, which many people avoid. Myristic acid is an important fatty acid, which the body uses to stabilize many different proteins, including proteins used in the immune system.



In this experiment, nutmeg cloves will be crushed (unless ground nutmeg provided) and the crude trimyristin will be extracted under reflux with diethyl ether. Due to the flammability of ether, any open flames are not permitted. The crude trimyristin will be purified through recrystallization and its purity checked by obtaining an IR spectrum. Finally, the pure trimyristin will be converted to myristic acid and glycerol by base-promoted hydrolysis (aka: saponification). The mechanism for this reaction is shown below – can you explain why the mechanism is called *base-promoted* and not *base-catalyzed*? The crude myristic acid will also be isolated and purified, and an IR spectrum will be taken to prove its purity. Looking at the structures given, how will an IR spectrum distinguish between the two molecules?

The base promoted mechanism is shown on the next page:



### Objective:

1. Extract and purify trimyristin from nutmeg. 2. Hydrolyze trimyristin to myristic acid. 3. Review of reflux, recrystallization, and IR techniques from 12AL. 4. Base-promoted hydrolysis / saponification mechanism to be learned.

### Procedure:

#### Isolation of Trimyristin

1. Set up a macro reflux apparatus; use a hot water bath for your heat source.
2. Measure out 1.5 grams of ground nutmeg into your reflux round bottom flask.
3. Add 7.5 mL of diethyl ether and a boiling chip.
4. Heat to a gentle reflux (low boil; you should see a vapor ring in bottom part of condenser!) and then continue to reflux for 45 minutes. Don't skimp on time - it takes awhile to unlock the trimyristin from the nutmeg!
5. After heating, let apparatus cool till warm to touch.

6. Use your micro- vacuum filtration set-up to filter the solution – WARM your plastic filtering funnel in warm water prior to filtering so that the trimyristin does not crystallize before it has a chance to filter into the flask.
7. Add a small amount of ether (1-2mL) to your reflux round-bottom flask to dissolve any remaining trimyristin that might be stuck to the flask and pour this over the residue in the filtering funnel.
8. Save the filtrate (filtrate = the solution that filtered thru and is in the flask).
9. Pour the filtrate into a small beaker and gently evaporate the ether (using a warm water bath) to crystallize your crude trimyristin.
10. Recrystallize your crude trimyristin using 95% ethanol in order to obtain pure trimyristin. Remember, you should be confident in Recrystallization from 12A! Review how to recrystallize if necessary!
11. Take an IR of your pure trimyristin. To take an IR of a solid product, dissolve a small amount of solid in dichloromethane (methylene chloride); pipette this solution into the IR trough; blow gently on it to evaporate the CH<sub>2</sub>Cl<sub>2</sub> leaving a solid thin film. (SAVE THE REMAINING TRIMYRISTIN FOR HYDROLYSIS)
12. Analyze your IR – all bonds and their wavenumbers must be labeled directly on the IR spectrum; also include structure of the molecule.

### **Hydrolysis of Trimyristin to Myristic Acid**

1. Measure out approximately 60 mg ( $\pm$  5 mg) of your trimyristin.
2. Transfer it to a clean round bottom flask. Add 2 mL of 6 M NaOH, 2 mL of 95% ethanol, and a boiling chip. If you have less than 60 mg of trimyristin, scale the hydrolysis down by a factor of 2 (halve the amounts of all reactants – trimyristin, NaOH, and ethanol - but keep the reaction time the same).
3. Reflux the solution gently in a warm water bath for 45 minutes. Overheating will cause the ethanol to evaporate. Note the approximate level of liquid in the flask and if necessary, replace any ethanol that is lost to evaporation simply by pipetting ethanol down the column during reflux.
4. After the hydrolysis has proceeded for 45 minutes, allow the flask to cool to room temperature and then pour the solution into a 50 mL beaker containing 8 mL of DI water.
5. In the hood, add dropwise with stirring, 2 mL of 6M HCl – what is the purpose of the HCl? Myristic acid should precipitate.

6. Cool the beaker in ice water for 10 min or more to maximize your yield of crystals.
7. Collect the solid by micro vacuum filtration while rinsing the solid with cold dl water. Dry your product thoroughly.
8. Take an IR of your myristic acid.
9. Analyze your IR – all bonds and their wavenumbers must be labeled directly on the IR spectrum; also include structure of the molecule.

Material adapted from: <http://www.multivitamin-s.com/myristicacid.html>;  
<http://www.chemistry.mtu.edu/pages/courses/organic/manuals/2411/Tri/Tri-09.pdf>  
<http://www.chem.umass.edu/~samal/267/trimyristin.pdf>

## 12BL Prelab Experiment 2: Extraction & Saponification of Trimyristin from Nutmeg

1. Give the **line bond structure** (not condensed as shown) of Trimyristin. What type of functional group is trimyristin?
2. Trimyristin is the triglyceride of myristic acid. Give the **line bond structure** (not condensed as shown) of myristic acid. What type of functional group is myristic acid?
3. Show the base-promoted hydrolysis **mechanism** of methyl acetate. Be neat and clear in all structures, arrows, charges, etc...
4. What bonds & their corresponding wavenumbers will trimyristin and myristic acid have in common on their IR spectrums? How will they differ?  
(use backside to answer)

## 12BL Postlab Experiment 2: Extraction & Saponification of Trimyristin from Nutmeg

Attach your analyzed IRs – all bonds and wavenumbers should be labeled with structure of product on IR.

1. Trimyristin can be hydrolyzed in base, NaOH, to form myristic acid. Write a **balanced chemical reaction** (not mechanism). Trimyristin should be shown in line/bond form.
  
2. Why is the proper term - “base-promoted hydrolysis” and not – “base-catalyzed hydrolysis? Be clear.
  
3. Research acid-catalyzed hydrolysis in your lecture textbook. Show the acid-catalyzed hydrolysis **mechanism** of methyl acetate. Be neat and clear in all structures, arrows, charges, etc...