Chem 210, Winona State University, Fall 2024

EXPERIMENT 9

Lipids - Isolation of Cholesterol and Trimyristin

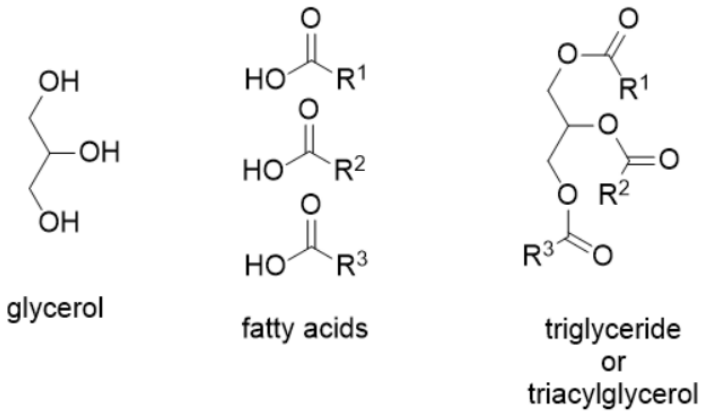
Adapted from F. A. Bettleheim and J. M. Landesburg, *Laboratory Experiments for General, Organic, and Biochemistry*, 3rd Ed., p. 417.

Materials Needed

1 g gallstones	2 grams powdered nutmeg	6 mL methanol
15 mL 2-butanone	20 mL hexane	10 mL acetone
50 mL Erlenmeyer flask	50 mL beaker	Hirsch or Buchner Funnel
hot plate	150 mL beaker	Watch glass and spatula

Background

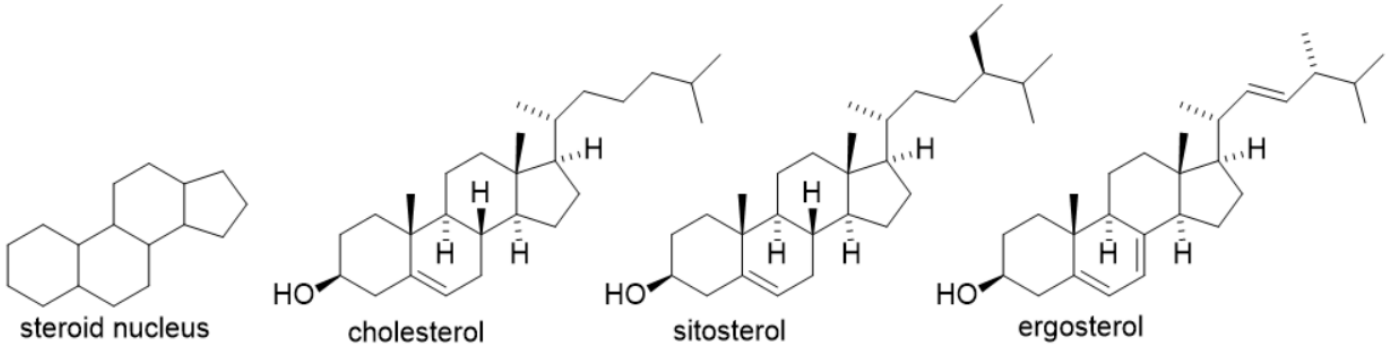
Lipids are biomolecules that are insoluble in water. The most commonly encountered lipids are the fats found in meats (e.g., lard and beef tallow) and the oils obtained from plants (e.g., soybean oil, olive oil, etc). These fats and oils are triesters of **glycerol** with three **fatty acids**, which are known as *triglycerides or triacyl glycerols*.



Naturally occurring animal or vegetable fats and oils are nearly pure triglycerides. The fatty acid chains can vary in length, generally between 12 and 18 carbons. The chains often incorporate alkene double bonds, which when present represent units of unsaturation. The unsaturation number (number of double bonds) plays a big factor in the physical and biological properties of the triglyceride. For example, a high degree of unsaturation results in a lower melting point. Hence, unsaturated triglycerides are oils at room temperature. Triglycerides that are mostly saturated have higher melting points and are solid fats at room temperature. The fatty acid chains can vary greatly, and frequently, represent a triglyceride that may be soft at room temperature, thereby forming a consistency between a fat and an oil.

Triglycerides that contain three identical fatty acids are unusual but one that is found in nature is trimyristin, which is essentially the only triglyceride present in the seeds of the fragrant nutmeg tree (*Myristica fragrans*). (These seeds when ground afford the spice known as nutmeg.) Trimyristin is the triester of three myristic acid molecules with glycerol so it is the structure shown above with $R^1 = R^2 = R^3 = -(CH_2)_{12}CH_3$. Because it lacks alkene double bonds, trimyristin is a fully saturated triglyceride causing it to be a solid at room temperature. The overall lack of polarity in the molecule causes it to be water insoluble but very soluble in nonpolar organic solvents such as hexane.

Another type of lipid is a group of compounds called the *steroids*. These large molecules always contain three cyclohexane rings and one cyclopentane ring fused together in the same manner forming what is known as the steroid nucleus or backbone. Perhaps, the most well-known steroid is cholesterol. Cholesterol is a key compound needed for cell membranes in all animals. Interestingly, plants and fungi use the slightly different steroids, sitosterol and ergosterol. Notice that these three steroids are all alcohols due to the presence of the OH group. However, the very large number of non-polar C-C and C-H bonds overwhelms the hydrophilic nature of the OH groups and makes these molecules water insoluble.



Although high blood levels of cholesterol are associated with heart disease and arteriosclerosis, it is an important constituent in the membranes of cells. It is also found in high concentration in the brain and nerves. Metabolically, it is an important precursor to vital hormones that the body needs, including the sex hormones, adrenaline, and cortisol, as well as the role it plays in bile salts in the gall bladder. Another condition that is caused by high levels of cholesterol is the formation of gallstones, which are essentially pure cholesterol which has crystallized inside a person's gall bladder.

PROCEDURE

Part I. Isolation of cholesterol

1. Set up a hot water bath (set hot plate to ~5) in the hood. Weigh 1.0 g of crushed gallstones into a dry 50 mL Erlenmeyer flask. Record the mass to the third decimal place.
2. To the flask, add 10 mL of 2-butanone.
3. Place a plastic short-stem funnel fitted with a fluted filter paper into the neck of the flask and heat the flask on the hot water bath. (The reason the funnel and filter paper are included in this step is because they need to be warmed up prior to the next step.) Monitor the liquid level in the flask. If it decreases due to 2-butanone boiling out, then remove the flask from the hot plate and add more.
4. While the mixture is hot, place the funnel and filter paper over a 150 mL beaker and quickly pour the mixture through it. The cholesterol, soluble in 2-butanone, will pass through the funnel leaving impurities behind.
5. To the colorless (or pale yellow) solution, add 4 mL of methanol. In the hood, reheat to boiling and then remove the beaker from the hot plate.
6. Use a Pasteur pipet to add distilled water one drop at a time until the solution first becomes cloudy. Allow the solution to cool to room temperature. Then cool it in an ice bath for 5 minutes.
7. Set up a Buchner funnel with a vacuum flask to filter the solids from the solution. (Revisit the part on vacuum filtration in experiment 3 (Alum) if you do not recall how to do this.)
8. Continue pulling air through the collected solid and wash it by pouring 2 mL of *ice-cold* methanol over it in the filter. Continue to vacuum until the crystals are mostly dry.
9. Place the crystals on a pre-weighed watch glass to air dry. When the crystals are dry, record the weight of them on the watch glass.
10. Snap a picture of your crystals and then discard them in the waste container.

Isolation of trimyristin

1. Weigh a dry 50 mL beaker.
2. Add 2.0 grams of powdered nutmeg to the beaker. Record the mass to the third decimal place.
3. Add 10 mL hexane to the beaker and warm gently on a hot plate (set between 2 and 3) in the hood. During the heating process, swirl the beaker gently to mix the contents and cover the beaker with a watch glass to prevent evaporation. Let it sit on the hot plate for about 10 min with occasional swirling.
4. Gravity filter the mixture using a fluted filter paper and collect the filtrate in a dry, pre-weighed, Erlenmeyer flask.
5. Use a spatula to carefully scrape the residue from the filter paper into the 50 mL beaker and repeat steps 3-4. The combined volume of the two filtrations from steps 5 and 6 should be about 20 mL.
6. In the hood, use a hot water bath to heat the flask containing the filtrate. The goal here is to evaporate off most of the hexane solvent. It is best to clamp the flask to keep it secure in the water bath. Be careful not to allow any water to get into your flask!
7. When the liquid in the flask is mostly gone, remove it and allow the contents to air dry and cool for another three minutes.
8. Add 5 mL of acetone to the oily trimyristin and place it in the hot water bath. As soon as the acetone starts to boil remove the flask from the bath and set it on the bench top.
9. Once the flask has cooled to near room temperature, place it in an ice bath to maximize the growth of trimyristin crystals.
10. Collect the crystals using vacuum filtration on a small Buchner funnel. Leave the vacuum on for 5-10 minutes to facilitate drying.
11. Transfer the crystals to a pre-weighed watch glass and determine the mass.
12. Snap a picture of your crystals and then discard them in the waste container.

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DATA AND OBSERVATIONS

Names _____ _Section _____ Date _____

Isolation of cholesterol

1. Mass of gallstones taken _____

2. Observations on gallstones:

3. Observations during procedures

4. Mass of empty watch glass _____

5. Mass of watch glass and cholesterol _____

6. Mass of cholesterol obtained _____

7. % Recovery of cholesterol _____
   (show your work below)

8. Observations on obtained cholesterol:

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Isolation of Trimyristin

1. Mass of Nutmeg taken _____

2. Observations on nutmeg:

3. Observations during procedures

4. Mass of empty watch glass _____

5. Mass of watch glass and trimyristin _____

6. Mass of trimyristin obtained _____

7. % Recovery of trimyristin _____
   (show your work below)

8. Observations on obtained trimyristin:

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REPORT

Names _____ _Section _____ Date _____

Results Table

Compound	Structure	Observations	% Recovery

Attach pictures of your obtained products to the end of this report.

QUESTIONS – TYPE UP AND ATTACH YOUR ANSWERS TO THIS REPORT SHEET

1. One reason for not obtaining 100% recoveries in this experiment is the fact that neither nutmeg or gallstones are 100% pure. In other words, gallstones are not 100% cholesterol and nutmeg is not 100% trimyristin. Do some research and list some other compounds That are present in each of these natural substances.

2. Look up the literature melting points and boiling points (in °C) for both cholesterol and trimyristin. Make a table that lists these data and give your reference(s).

3. What is the difference in intermolecular interaction, in cholesterol versus trimyristin, that gives such a wide contrast in their melting points? Discuss both the intermolecular forces and the packing ability of each molecule. Be specific.

4. Draw the structure of a triglyceride molecule which contains the following fatty acid residues: myristic acid, oleic acid, and linolenic acid.

5. Would you expect the triglyceride in question 4 to have a lower or higher melting point than trimyristin? Explain.