EXPERIMENT 8

Lipids - Isolation of Cholesterol and Trimyristin

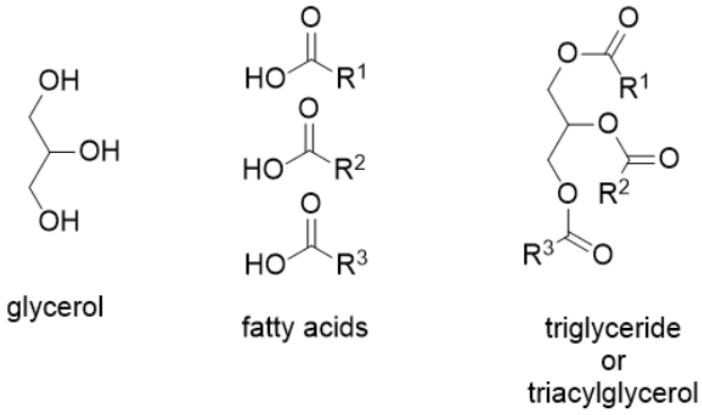
Adapted from F. A. Bettleheim and J. M. Landesburg, *Laboratory Experiments for General, Organic, and Biochemistry*, 3rd Ed., p. 417.

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

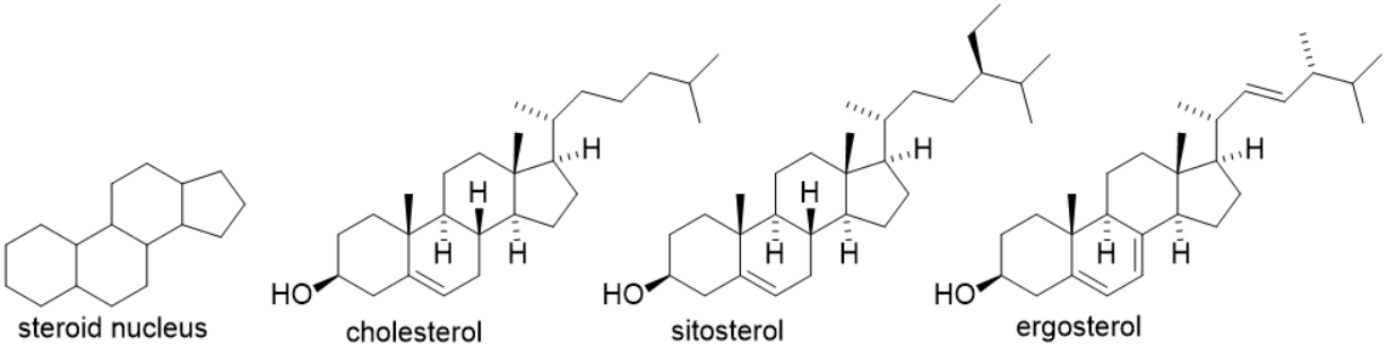
Lipids are biomolecules that are insoluble in water. The most commonly encountered lipids are the fats found in meats (e.g., lard and beef tallow) and the oils obtained from plants (e.g., soybean oil, olive oil, etc). These fats and oils are triesters of **glycerol** with three **fatty acids**, which 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 is 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 out a precise amount (around a gram) of crushed gallstones and transfer to a dry 50 mL Erlenmeyer flask. Record the weight used.
2. In the hood, add 10 mL of 2-butanone. Place a plastic funnel fitted with filter paper into the neck of your pre-weighed flask.
3. In the hood, boil gently on low heat for 3 minutes. (The boiling will help get the paper hot for hot filtration.) Be aware of the butanone level in the flask. If it drops due to boiling, remove the flask from the hot plate and add more.
4. While the mixture is hot, hold the funnel 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. Drop wise, add distilled water until the solution is cloudy. Allow the solution to cool to room temperature. Then cool it in an ice bath for 5 minutes.
7. Set up a Hirsch funnel with a vacuum flask to filter the solids from the solution. (Revisit the use of a Hirsch funnel in Experiment 2 (Extraction) if you do not recall how to do this.
8. Continue vacuuming the solids and wash the solid cholesterol by pouring 2 mL of *ice-cold* methanol over the solids in your 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. Dispose of your crystals in the marked container.

Isolation of trimyristin

1. Weigh a dry 50 mL beaker.
2. Add about 2 grams of powdered nutmeg to the beaker. Record the exact weight of the nutmeg and the beaker.
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. Caution: hexane is flammable. Make sure there are no open flames nearby.
4. Filter the mixture through a dry 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 on a hot plate to slowly evaporate the hexane from the filtrate in the flask by placing your flask in the bath. You may want to clamp your flask to keep it steady in the water bath. Be careful not to allow any water to get into your flask.
7. When the flask is nearly dry, remove it and allow the contents to air dry and cool for another three minutes.
8. Add 10 mL of acetone to the oily trimyristin and place it in the hot water bath. When the volume of the solution is about 5 mL, remove the flask from the hot water bath and place it in an ice bath to allow the trimyristin to crystallize out of solution.
9. If the flask appears completely dry, weigh the flask and record the mass of the pure trimyristin. If there is acetone remaining in the flask, filter the contents using a small Hirsch funnel. Place the crystals on a pre-weighed watch glass and weigh the contents.
10. Dispose of your crystals in the marked container.

Chem 210, Winona State University, Fall 2022

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PROCEDURE AND REPORT SHEET

Names _____ _Section _____ Date _____

Isolation of cholesterol

1. Mass of gallstones used _____
2. Mass of empty watch glass _____
3. Mass of watch glass and cholesterol _____
4. Mass of cholesterol _____

5. Description of appearance of cholesterol:

6. Percent recovery of cholesterol. Assume that gallstones are essentially 100% cholesterol. Show your work!

Isolation of trimyristin

1. Mass of empty watch glass _____
2. Mass of watch glass and trimyristin _____
3. Mass of trimyristin _____
4. Description of appearance of trimyristin:

5. Percent recovery of trimyristin. Assume that nutmeg is essentially 100% trimyristin. Show your work!

QUESTIONS

1. Cholesterol can form well-defined crystals in gallstones. What kind of intermolecular interactions would provide the most stable structure of cholesterol in the crystals?

2. Look up the literature melting points for both cholesterol and trimyristin. Record them.

Cholesterol _____ Trimyrsitin _____

Reference_____ Reference_____

3. What is the difference in intermolecular interaction, in cholesterol versus trimyristin, that gives such a wide contrast in their melting points? 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 5 to have a lower or higher melting point than trimyristin? Explain.