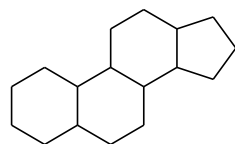

Experiment 16 – Analysis of Lipids

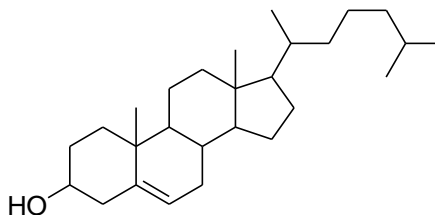
Discussion

Lipids are chemically heterogeneous mixtures. The only common property they have is their insolubility in water. We can test for the presence of various lipids by analyzing their chemical constituents. Foods contain a variety of lipids; most important among them are fats, complex lipids, and steroids. Fats are triglycerides, esters of fatty acids and glycerol. Complex lipids also contain fatty acids, but their alcohol may be either glycerol or sphingosine. They also contain other constituents such as phosphate, choline, ethanolamine, or mono- to oligosaccharides. An important representative of this group is lecithin, a glycerophospholipid, containing fatty acids, glycerol, phosphate, and choline. The most important steroid in food is cholesterol. Different foods contain different proportions of these three groups of lipids.

Structurally, cholesterol contains the steroid nucleus that is the common core of all steroids:



Steroid nucleus



Cholesterol

There is a special colorimetric test, the Lieberman–Burchard reaction, which uses acetic anhydride and sulfuric acid as reagents, that gives a characteristic green color in the presence of cholesterol. This color is due to the –OH group of cholesterol and the unsaturation found in the adjacent fused ring. The color change is gradual: first it appears as a pink coloration, changing later to lilac, and finally to deep green.

A test that differentiates between cholesterol and lecithin is the acrolein reaction. When lipids containing glycerol are heated in the presence of potassium hydrogen sulfate, the glycerol is dehydrated, forming acrolein, which has an unpleasant odor. Further heating results in polymerization of acrolein, which is indicated by the slight blackening of the reaction mixture. Both the pungent smell and the black color indicate the presence of glycerol and therefore fat and/or lecithin. Cholesterol gives a negative acrolein test.

Procedure**The Acrolein Test for Glycerol**

1. Place 1 g of potassium hydrogen sulfate, KHSO_4 , in each of seven clean and dry test tubes (100 x 13 mm). Label them 1 through 7. To the first test tube, add a few grains of pure lecithin; to test tube #2, add a few grains of cholesterol. To test tubes #3 - #6, add one separate drop (which measures approximately 0.1 g) of glycerol (to #3), corn oil (to #4), butter (to #5), and egg yolk (to #6). To the seventh test tube, add a few crystals of sucrose.
2. Set up your Bunsen burner near a hood. **Caution:** Perform this test near the hood due to the pungent odor of the acrolein. When asked to smell the test tubes, do not inhale the fumes directly. Smell the test tubes by moving them sideways under your nose or by wafting the vapors toward your nose with a cupped hand.
3. Gently heat each test tube, one at a time, over a Bunsen burner flame, shaking it continuously from side to side. When the mixture melts and slightly blackens, you will notice the evolution of fumes. Stop the heating. Carefully smell the test tubes; pay attention to the **Caution** above. A pungent odor, resembling burnt hamburgers, is a positive test for glycerol. Sucrose in the seventh test tube will also be dehydrated and give a black color. However, its smell is different and thus is not a positive test for acrolein. Do not overheat the test tubes, for the residue will become hard, making it difficult to clean the test tubes. Record your observations on your data sheet.

Lieberman–Burchard Test for Cholesterol

1. Take six clean and dry test tubes (150 x 18 mm). Label them 1 through 6. Place a few grains of your cholesterol and lecithin (separately) in the first two test tubes. Similarly, add about 0.1 g samples of glycerol, corn oil, butter, and egg yolk (separately) to the other four test tubes.
2. Transfer 3 mL of chloroform and 1 mL of acetic anhydride to each test tube. Finally, *carefully* add 1 drop of concentrated sulfuric acid to each mixture. Mix the contents and record the color changes, if any. Wait 5 min. Record again the color of your solutions. Record your observations on your data sheet.

Name: _____

Section: _____

Data and Calculations for Experiment 16

<i>Test</i>	<i>Cholesterol</i>	<i>Lecithin</i>	<i>Glycerol</i>	<i>Corn Oil</i>	<i>Butter</i>	<i>Egg Yolk</i>	<i>Sucrose</i>
A. Acrolein							
a. Odor							
b. Color							
c. Conclusions							
B. Lieberman–Burchard							
a. Initial color							
b. Color after 5 min							
c. Conclusions							

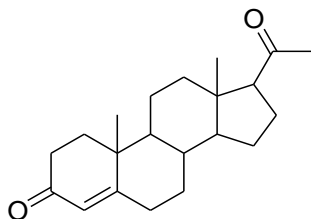
Questions

- From your results, what is present in corn oil? Is it a pure triglyceride?
- Based on the intensity of the color in your test for cholesterol, which food showed the most cholesterol present? Which food showed the least cholesterol present?

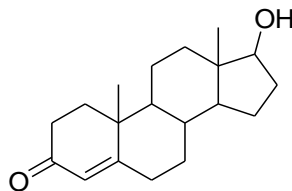
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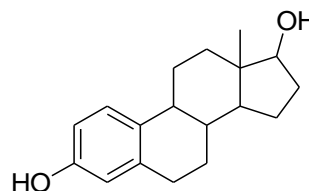
3. Consider the steroid structures shown below. Would any of these structures give a positive Lieberman-Burchard test? Briefly explain.



Progesterone



Testosterone



Estradiol

4. Cholesterol is an alcohol that can dehydrate to form a carbon-carbon double bond. Draw the structure cholesterol forms upon dehydration. Would this dehydration compound give a positive Lieberman-Burchard test? Briefly explain.