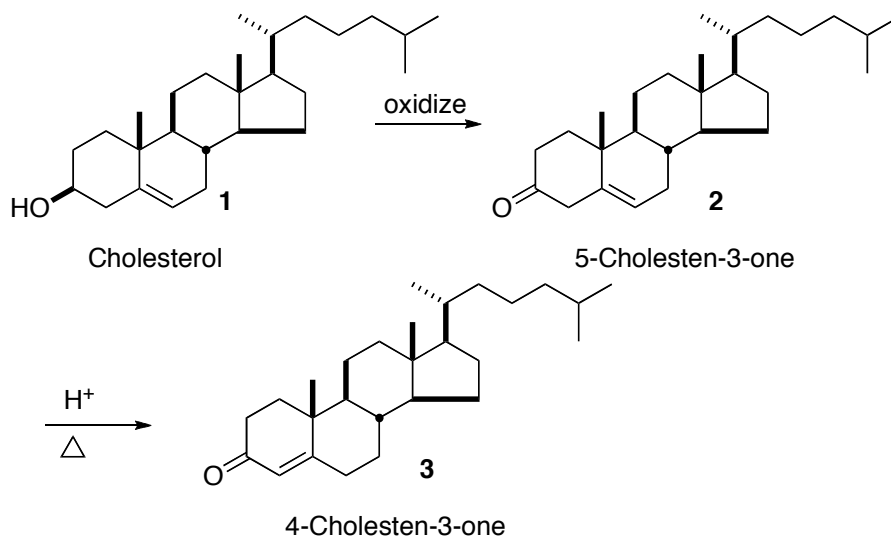


Isolation of Cholesterol from Egg Yolk

Preparation: Bring a hard-boiled egg yolk to lab!

Cholesterol (**1**) is a major component of cell membranes. An egg yolk contains about 200 milligrams of cholesterol, much of it bound as complex lipid. In this experiment, you will purify cholesterol from an egg yolk. You can then choose to either crystallize the cholesterol to purity (option #1), or carry out the oxidation and conjugation (option #2), leading to 4-cholestene-3-one **3**.



Procedure: In a 250 mL round bottom flask, combine a hard-boiled egg yolk, 1 g of K_2CO_3 , 5 g of sand, and 10 mL of MeOH. Grind together until it is smooth – it will look like soft scrambled eggs. Add 20 mL of cyclohexane, stir thoroughly – it will look like corn meal mush – then warm to reflux. Rotovap off the solvent. While the solvent is rotovapping, prepare a chromatography column with 15 g (30 mL) of flash silica gel and a layer of sand on top, and have fifteen test tubes ready to take fractions. Also prepare a mixture of 30 mL of EtOAc and 170 mL of petroleum ether.

To the 250 mL round bottom flask with the evaporated egg mixture, add 30 mL CH_2Cl_2 , and stir thoroughly. Add the CH_2Cl_2 solution (not the egg mixture!) to the top of the column, and let the solvent go down on its own. When all the CH_2Cl_2 is down, rinse the inside top of the column with a little of the EtOAc/pet ether mixture.

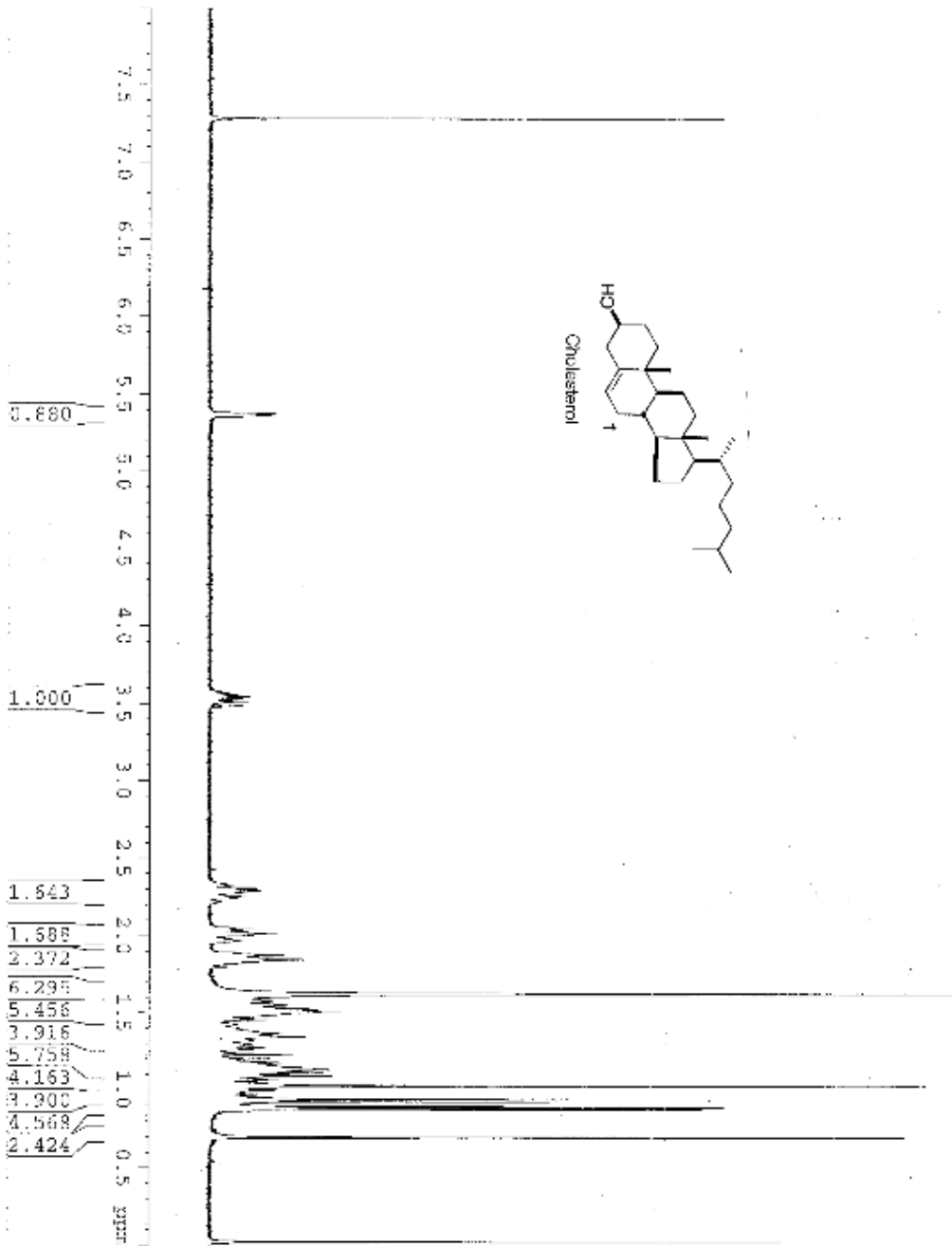
By this time, the solvent should have begun to drip out of the bottom of the column. Add more of the EtOAc/pet ether mixture to the top of the column, and apply gentle air pressure to the column as you collect 10 mL fractions. You should collect 15 fractions. Check the fractions by thin layer chromatography. Usually, the cholesterol comes in fractions 6-10. Rotovap the cholesterol fractions in a tared round bottom flask. You should be rewarded with iridescent rosettes of the product. Record the weight and the melting point.

Option #1: Cholesterol forms a specific 2:1 complex with oxalic acid. Take up your crude cholesterol in 5 mL of 1,2-dichloroethane in a 50 mL Erlenmeyer flask. Add 80 mg of oxalic acid, and heat to reflux. Let the flask cool. After twenty minutes (clean up the lab!), swirl the flask in ice water. The contents should gel into a mush of crystals. Vacuum filter with 1,2-dichloroethane, suck dry, and spread out to dry. Take up the white residue in a 50 mL Erlenmeyer flask with 5 mL of water. Heat to reflux, chill in ice water, and vacuum filter. This should give pure white cholesterol, mp = 141-143 °C. Record the melting point and weight. Yields are often better if the complexation with oxalic acid is allowed to go overnight.

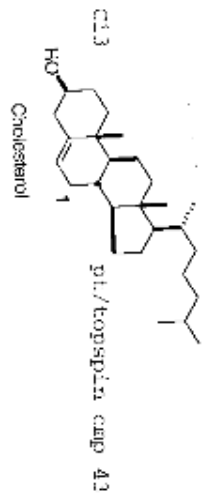
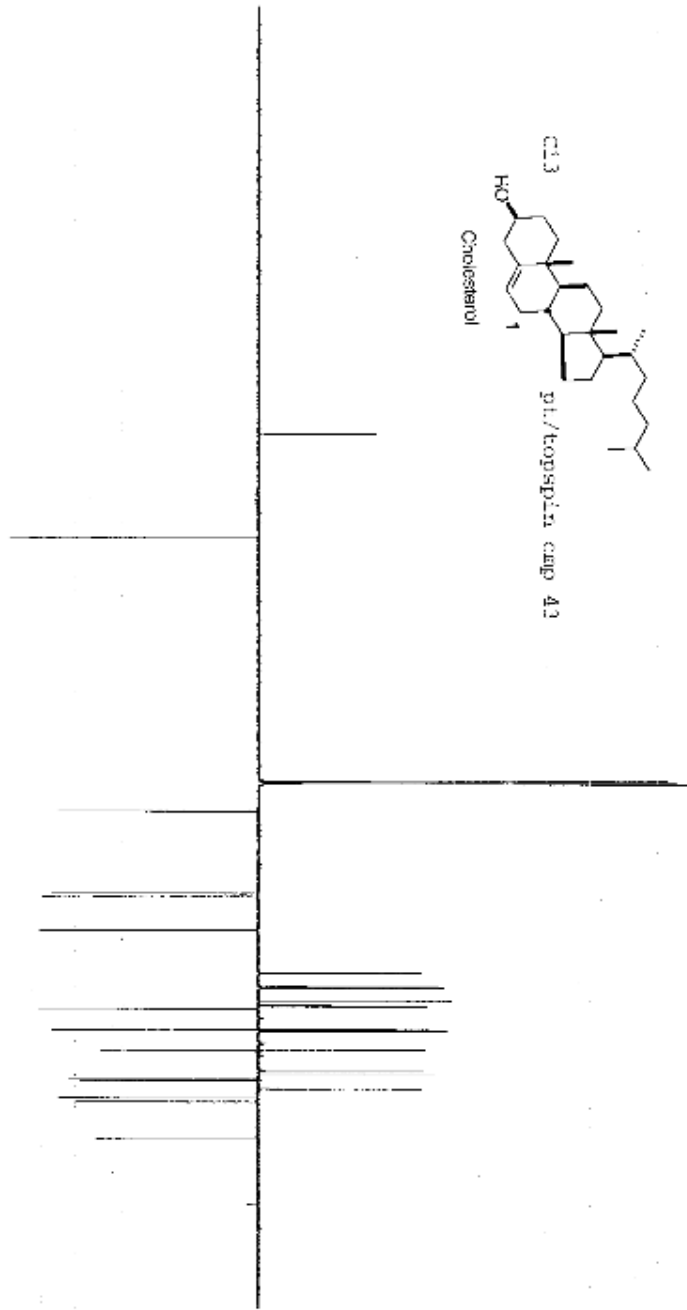
Option #2: Take up the crude cholesterol in 5 mL of CH₂Cl₂ in a 50 mL round bottom flask. Spot a TLC plate in the left lane and the middle lane with the CH₂Cl₂ solution. Add 0.5 mL of Dess-Martin reagent in CH₂Cl₂, and warm briefly. Spot the solution in the middle and the right lanes of the same TLC plate, and develop in 1:4 EtOAc/pet ether. If a lot of starting material still remains, warm the solution again, and check it again. When the starting material is almost all converted, save a little of the CH₂Cl₂ solution. Rotovap the rest of the solution, add 8 mL of acetone and 20 mg *p*TsOH, and warm it again to reflux. Spot the TLC plate middle and right, and develop it in 1:4 EtOAc/pet ether. This time, before you visualize the TLC plate with I₂, check it with the UV light. You should see a UV spot for cholestenone. TLC R_f's in 1:9 EtOAc/pet ether: cholesterol = 0.28, 5-cholesten-3-one = 0.60, 4-cholesten-3-one = 0.41.

To purify the 4-cholesten-3-one, add 2 g of flash silica gel to the acetone solution and rotavap it. Build a dry column with 10 grams (20 mL) of flash silica gel. Tap it to settle the silica gel, then add the dry powder with your reaction mixture, and then sand on top. Elute with a mixture of 2 mL of EtOAc in 100 mL of petroleum ether, then with a mixture of 5 mL of EtOAc and 95 mL of petroleum ether. Take 10 mL fractions, and check them by TLC. Evaporate the fractions containing 4-cholesten-3-one in a tared round bottom flask, and record the weight and melting point.

If desired, the 5-cholesten-3-one **2** (before adding the acetone and *p*TsOH!) can be isolated following the same procedure, but with 1 mL of EtOAc in 100 mL of petroleum ether, followed by 2 mL of EtOAc in 100 mL of petroleum ether.



200 180 160 140 120 100 80 60 40 20 0 ppm



- 140.750
- 130.039
- 128.731
- 128.705
- 127.984
- 121.759
- 77.353
- 77.037
- 76.719
- 71.818
- 62.113
- 56.759
- 56.126
- 50.105
- 42.315
- 42.288
- 39.942
- 39.769
- 39.522
- 37.244
- 36.505
- 36.188
- 35.805
- 34.044
- 31.920
- 31.890
- 31.651
- 29.788
- 29.732
- 29.357
- 29.228
- 29.141
- 28.257
- 28.058
- 27.874
- 27.192
- 24.898

0.50

