Isolation of Cholesterol from an Egg Yolk

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Supporting Information

ABSTRACT: A simple procedure for the isolation of the cholesterol, by hydrolysis and extraction followed by column chromatography, is described. The cholesterol can be further purified by complexation with oxalic acid. It can also be oxidized and conjugated to cholestenone. The source of the cholesterol is one egg yolk, which contains about 200 mg of lipid-bound cholesterol. This experiment is appropriate for an undergraduate organic chemistry laboratory and can be completed in 3 h.

KEYWORDS: Second-Year Undergraduate, Laboratory Instruction, Organic Chemistry, Hands-On Learning/Manipulatives, Alcohols, Aldehydes/Ketones, Chromatography, Natural Products, Oxidation/Reduction, Steroids

Decades ago, the isolation and purification of cholesterol, 1 (Figure 1), from human gallstones was a standard organic lab experiment. Current biosafety concerns prohibit students from handling such human remains. In contrast, egg yolks are safe and are readily available, and one egg yolk contains about 200 mg of cholesterol. Although several experiments centered on egg yolks have been reported in this Journal, the isolation of cholesterol from the egg yolk had not been addressed.

EXPERIMENT AND RESULTS

A simple procedure for the isolation and purification by column chromatography of cholesterol from a hard-boiled egg yolk is described. The key to this procedure is a quick saponification of the yolk lipid with methanol/K₂CO₃, followed by azeotropic removal of the methanol by evaporation with cyclohexane. The cholesterol after chromatography is pure enough to characterize by melting point. It can then be brought to high purity by reversible formation of its complex with oxalic acid. Typically, a student obtains 50–60 mg of cholesterol after the initial chromatography column and 15–30 mg after the oxalic acid purification. A rapid protocol has also been devised, using the modern Dess–Martin reagent, for the oxidation of 1 to the ketone 2, with subsequent conversion of 2 to the enone 3. The procedures for these transformations and the ¹³C and ¹H NMR spectra for 1, 2, and 3 are included in the Supporting Information.

This experiment has been performed by 12 sections of approximately 24 first-semester organic students without difficulty. All of the students in a section isolated the cholesterol from the egg yolk and purified it by column chromatography. Then, half of the students further purified the cholesterol with oxalic acid complexation and the other half of the students reacted the cholesterol to form the ketone and the enone. Students were given the ¹³C and ¹H NMR spectra for 1, 2, and 3. The two options take about the same elapsed time and the entire lab can be completed in 3 h.

HAZARDS

When dealing with any of the solvents or reagents in this lab, standard laboratory safety precautions apply. Dess–Martin reagent is a skin irritant. Methanol, cyclohexane, and acetone are flammable and must be kept away from an open flame. Direct ingestion or inhalation of all solvents and reagents should be avoided. Dichloromethane and 1,2-dichloroethane are inhalation hazards, as is silica gel. These should be used in a well-ventilated hood. Silica gel, with a density of 0.5, is best measured using a graduated cylinder. Latex gloves should be used when handling iodine, which can cause skin stains and burns.

DISCUSSION

This experiment is best suited for an undergraduate organic chemistry lab. Starting from a hard-boiled egg yolk, the extraction and purification of cholesterol takes about 3 h. Students performing the procedure learn about natural product isolation, column chromatography, crystallization, oxidation, and reduction. There is also a strong biochemical correlation with this procedure, because cholesterol is an important component of cell membranes. In recovering their reaction products, students learn how to properly use separatory funnels, Buchner funnels, and the
rotary evaporator. Additionally, the students learn how to quickly purify and identify the products through recrystallization and melting point determination. As the experiment requires no unusual laboratory equipment or reaction conditions, the isolation of cholesterol is a simple yet instructional procedure for any undergraduate student to perform.

**CONCLUSION**

Apart from laboratory techniques, students also have the opportunity to research several current and important topics in organic chemistry. In addition to its importance both in biochemistry and in human disease, cholesterol is the key component of many liquid crystals, which are widely used in display technology. 10

**ASSOCIATED CONTENT**

# Supporting Information

Student handout; notes for the instructor; 13C and 1H NMR spectra. This material is available via the Internet at http://pubs.acs.org.

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**REFERENCES**


6 (6) For a flash chromatography procedure suitable for the undergraduate teaching laboratory, see Taber, D. F.; Hoerrner, R. S. Column Chromatography: Isolation of Caffeine. J. Chem. Educ. 1991, 68, 73–73. We have found it convenient to lightly pressurize the columns using a standard two-button pipette bulb.


