2. Epoxidation of Cholesterol

M. Jones: Oxiranes, 10.4a, Figure 10.27, pgs 452-455
Asymmetric (Sharpless) Epoxidation, 10.4b, pgs 859-866
Steroid Biosynthesis, 12.14, pgs 604-608
Chromatography, 4.9, p 177, 10.2, pgs 749-752

J.R. Mohrig, C.N. Hammond, and P.F. Schatz: Technique 17, 17.1 – 17.7, pgs 178 – 189

This procedure has been adapted from the microscale procedure described in the third edition of *Macroscale and Microscale Organic Experiments* by Kenneth L. Williamson (Houghton Mifflin, Boston, 1999).

Introduction

In this experiment, you will perform an epoxidation of an alkene using a peroxyacid. You will also be introduced to a new technique, chromatography, which will be used in later experiments.

Background

The epoxidation reaction is the formation of an oxirane (epoxide) from an alkene. There are different reagents which can be used to accomplish this transformation. Different reagent systems, including the Sharpless epoxidation of allylic alcohols using tartaric acid, have been used to affect this reaction stereospecifically. One of the more common reagents is a peroxyacid. A specific reagent used for this purpose is 3-chloroperoxybenzoic acid, more commonly know as *meta*-chloroperbenzoic acid (*M*CPBA). The mechanism is depicted in Figure 1.

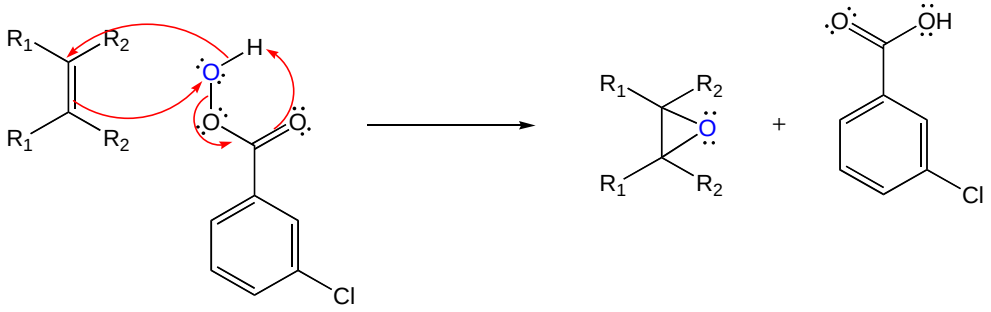
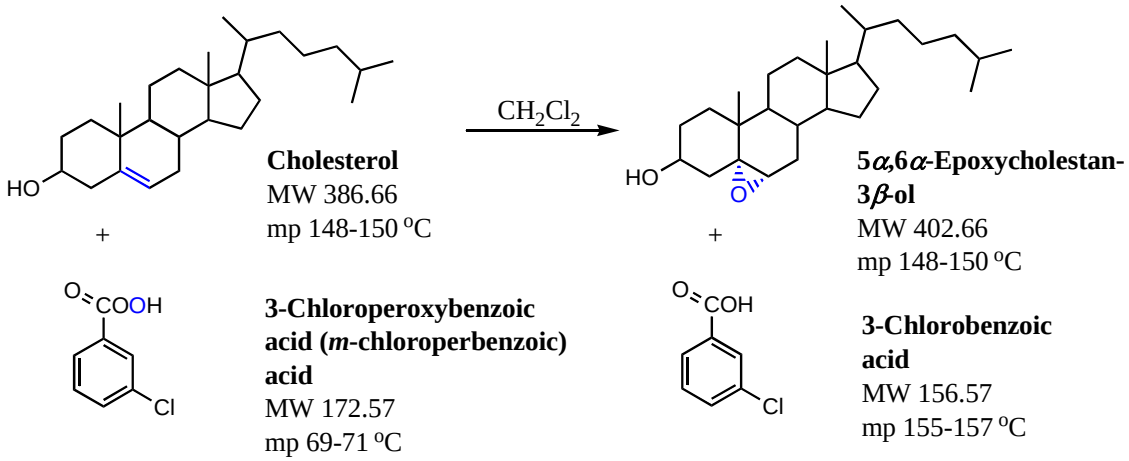


Figure 3. The mechanism for epoxidation of a generic alkene with *m*-CPBA.

The mechanism is concerted; therefore the epoxide retains the regiochemistry of the starting alkene.

In this laboratory experiment, you will be synthesizing an epoxide starting from cholesterol, which is an interesting molecule. All of the ring junctions are *trans* to one another and the groups are on the same side of the molecule.



Cholesterol
MW 386.66
mp 148-150 °C

3-Chloroperoxybenzoic acid (*m*-chloroperbenzoic) acid
MW 172.57
mp 69-71 °C

5α,6α-Epoxycholestan-3β-ol
MW 402.66
mp 148-150 °C

3-Chlorobenzoic acid
MW 156.57
mp 155-157 °C

Figure 3. The overall reaction of this experiment.

Cholesterol has had a lot of recent notoriety especially with respect with human health. A lot has been discussed about testing for "good" and "bad" cholesterol, whether we can decrease it, and what is the basis for some people to have "high" or "low" cholesterol. These topics are not going to be discussed in this laboratory but should be on our minds.

A new technique, which will be introduced in this laboratory, is chromatography. It is used as a separation and/or purification method, especially if one has more than one product, and the normal extraction and/or recrystallization processes do not work. It is a technique of practice and patience.

It was first introduced to it in Chapter 4 when enantiomers were discussed, and this topic will be further discussed in Chapter 15. In a nutshell, you have two different phases, a stationary (one that does not move and provides a scaffold) and mobile (one that moves, in your case a solvent) phase. The polarity of the compound and solvent as well as the compounds' molecular weights play into this separation process.

Experimental

Remember to add either a boiling stick or chips when heating a reaction.

Reaction.

Place 200 mg of cholesterol and 1 mL of methylene chloride (dichloromethane, CH_2Cl_2) into a small reaction tube and warm (if needed) to dissolve. Place 140 mg of 3-chloroperoxybenzoic acid (*m*-chloroperbenzoic acid) and 2 mL of methylene chloride into another small reaction tube and warm to dissolve. Let both tubes cool to room temperature, and then add the benzoic acid solution to the cholesterol solution dropwise via pipette. Add a boiling stone and then cap the reaction tube and heat for 30 minutes using a sand bath.

Making the Column.

The product will be separated from the starting materials and by-products using column chromatography. You will be preparing an alumina column using a micro-column. Obtain a micro-column and check that is fitted with a fritted disc (frit), column tip, and small funnel. Assemble the column, tip and funnel, insuring that the tip is completely flush with the end of the column. If the column appears to be dirty, clean it with a small amount of acetone.

Next, weigh out 3 g of alumina and pour into a small reaction tube and cap it. Obtain 30 mL of *tert*-butyl methyl ether and place it in a large reaction tube and cap it. Once your 30 min reaction time is finished, remove your reaction mixture from the sand bath and start wet loading your column.

Slurry Method for Packing the Column (Wet-Loading).

Secure your constructed column using a clamp and place a pre-weighed 50 mL Erlenmeyer flask beneath it. To your small reaction tube containing the alumina, add in *tert*-butyl methyl ether until the reaction tube is ¾ full and use a glass stirring rod to swirl the contents and allow it to settle. Use a glass pipette to transfer the alumina/ether mixture (called a slurry) to the column. Be sure to work steadily so the level of the ether in the column does not go below the level of the solid alumina. Add more ether to the small reaction tube if necessary. Once all (or most) of the alumina has been added, make sure the *tert*-butyl methyl ether solvent line does not go below the alumina in the column. Using the rubber end of your spatula, gently tap the side of the column so the top of the alumina is as level as possible. If you need to add additional ether before loading your sample, place the tip of the pipette against the side of the column, and allow the ether to gently run down the inside of the column to prevent disturbing the alumina. Your column is now packed and ready for use.

Note: You may reuse the *tert*-butyl methyl ether collected in the Erlenmeyer flask to keep the column from going dry until you are ready to load your sample.

Workup.

Your reaction product will be separated from the starting material and any by-products from the reaction by using column chromatography. Once the reaction mixture is cooled, it is ready to be added (loaded) to the column of alumina in *tert*-butyl methyl ether.

Before loading your product onto the column, allow the *tert*-butyl methyl ether to elute until it reaches ½ cm above the alumina. Then, pipette your liquid product gently onto the column while being careful not to disturb the alumina surface. Once the liquid layer is ½ cm above the alumina again, gently add more *tert*-butyl methyl ether to fill the column to the top (but below the cone). Do not allow the column to go dry! Continue eluting the product into the pre-weighed 50-mL Erlenmeyer flask until 30 mL of solution have been collected.

Add a boiling stick to the flask and concentrate the solution to dryness in the hood. Once cooled to room temperature, obtain a crude product mass. **(If the mass of product is less than 150 mg, then elute the column with another 10 mL of *tert*-butyl methyl ether.)** Recrystallize the resulting solid in acetone/water. Obtain the final mass of the recrystallized product and ask your TA to help you perform a Beilstein test.

Testing the Product.
Perform a Beilstein test to determine if your product contains chlorine. This test is performed by heating a copper wire in the flame of a Bunsen burner until no additional coloration of the flame is noticed. Next, the copper wire is cooled by dipping it into a beaker of deionized water. Then, dip the copper wire into your sample and heat in the flame again. Record any color change you observe.