Epoxidation of Cholesterol

from K. L. Williamson, Macroscale and Microscale Organic Experiments, 2nd Ed. 1994, Houghton Mifflin, Boston  p300 Rev 2/22/02

Prelab Note: In your chemical data table, be sure to account for the fact that the 3-chloroperoxybenzoic acid is only about 80% pure when calculating mmols of reactants.

PreLab Exercise

When milk is irradiated with UV light, the vitamin D content increases. What is the reaction that is taking place?

Introduction

This experiment allows you to carry out an epoxidation reaction on cholesterol, which is a representative of a very important group of molecules, the steroids. The rigid cholesterol molecule gives products of well-defined stereochemistry. The epoxidation reaction is stereospecific, and the product can be used to carry out further stereospecific reactions.

Cholesterol itself is the principal constituent of gallstones and can be readily isolated from them (see Exp't 110). The average person contains about 200 g of cholesterol, primarily in brain and nerve tissue. The closing of arteries by cholesterol leads to the disease arteriosclerosis (hardening of the arteries).

Certain naturally occurring and synthetic steroids have powerful physiological effects. Progesterone and estrone are the female sex hormones, and testosterone is the male sex hormone; they are responsible for the development of secondary sex characteristics. The closely related synthetic steroid norethisterone is an oral contraceptive, and addition of four hydrogen atoms (reduction of the ethynyl group to the ethyl group) and a methyl group gives an anabolic steroid, ethyltestosterone, a muscle-building steroid now outlawed for use by Olympic athletes. Fluorocortisone is used to treat inflammations such as arthritis. Ergosterol on irradiation with ultraviolet light is converted to vitamin D₂.
Much of our present knowledge about the stereochemistry of reactions was developed from steroid chemistry. In this experiment, the double bond of cholesterol is stereospecifically converted to the 5-, 6-epoxide. The _ designation indicates that the epoxide is on the backside of the molecule. A substituent on the topside is designated _. Study of molecular models reveals that the angular methyl group prevents topside attack on the double bond by the perbenzoic acid; hence the epoxide forms exclusively on the back, or _, side of the molecule.

Epoxides are formed most commonly by reaction of a peroxycarboxylic acid with an olefin at room temperature. It is a one-step cycloadition reaction:

The reaction is carried out in an inert solvent, dichloromethane, and the product is isolated by chromatography. The 3-chlorobenzoic acid, being polar, is adsorbed strongly onto the alumina, while the relatively nonpolar product is eluted easily by ether (use the ether in your **hood**). After removal of ether, the product is easily recrystallized from a mixture of acetone and water.

**Cautions**

Some peroxycarboxylic acids are explosive; the reagent used in the present experiment is a particularly stable and convenient peroxycarboxylic acid that is quite safe to handle.

**TLC**

You are required to run a TLC to monitor the progress of the reaction. Plates should have three spots (or lanes) on the origin: one for the main organic starting material that is being transformed, one for a cospot (starting material and the reaction mixture), and one for the reaction mixture.

**Synthesis of Cholesterol Epoxide**
Dissolve 194 mg of cholesterol in 0.8 mL of dichloromethane in a 10 x 100 mm reaction tube by gentle warming, then allow to cool to room temperature. Make a second solution by gently warming 117 mg of 80% 3-chloroperoxybenzoic acid (or 187 mg of 50% material) in 0.8 mL of dichloromethane and cool to room temperature also. Drop a 1/2" magnetic stirbar into the latter tube and add the cholesterol solution to this tube; mix the two solutions together. Cork loosely and place the stoppered reaction tube in a beaker of warm tap water at 35 to 45°C for 50 minutes with stirring or until the reaction is complete as shown by TLC analysis. The progress of the reaction can be followed by thin-layer chromatography on silica gel plates using diethyl ether (use the wet ether found in a supply bottle in each hood) as the eluent.

Isolation and Purification by Chromatography:

NOTE - The epoxide product is often isolated as a clear thick oily residue and not a crystalline solid. Do not throw non-crystalline product away by mistake. Dry 50 mL of diethyl ether (use the wet ether found in a supply bottle in each hood) over 2 or 3 tablespoons of solid Na₂SO₄ for 15 minutes with occasional swirling. Fill your 10 x 200 chromatography column with this ether and add 3 g alumina. Tap the column with a pencil while filling, to remove air bubbles and place a thin layer of sand on top. The reaction mixture is concentrated to ~ 0.2 mL by blowing down with a stream of nitrogen and then pipetted onto this column as in Chapter 8 of the Lab Guide. The 3-chlorobenzoic acid will be adsorbed strongly by the alumina. The product is eluted with 30 mL of diethyl ether collected in a tared (previously weighed) 50-mL Erlenmeyer flask. The ether can flow through the column by gravity or can be forced out by applying nitrogen pressure (flash chromatography). Ask your TA to demonstrate. It should not be necessary, but you can monitor the chromatography by TLC, use iodine visualization of spots as cholesterol and its epoxide don’t show up under UV.

The diethyl ether is removed by allowing it to evaporate in the hood until the next lab. Label the flask. The residue should weigh more than 150 mg. If it does not, pass more ether through the column and collect the product as before. Dissolve the product in 1.5 mL of warm acetone, and using a Pasteur pipette, transfer it to a reaction tube. Add 0.2 mL of water to the solution, warm the mixture to bring the solid into solution, and then let the tube and contents cool slowly to room temperature. Cool the mixture in ice, and collect the product on a Hirsch funnel. Press the solid down on the filter to squeeze solvent from the crystals, and then wash the product with 0.25 mL of ice-cold diethyl ether. Spread the product out on a watch glass to dry. Determine the weight and melting point of the product, and calculate the percentage yield.

Analysis
In addition to TLC analysis, you may be instructed to analyze your final product by NMR or MS. Analyze your sample according to your assignment sheet and the instructions on Sample Preparation in the Lab Guide.
If you are required to run a ¹H NMR, use the 400 MHz instrument; consult a TA.

Cleaning Up
Place dichloromethane solutions in the halogenated organic solvents container and organic solvents in the organic solvents container. The alumina should be placed in the hazardous waste container. If it should be necessary to destroy 3-chloroperoxybenzoic acid, add it to an excess of an ice-cold solution of saturated sodium bisulfite in the hood. A peracid will give a positive starch/iodide test (blue-purple color).

Post Lab Questions
1. Draw the structure of 3-chloroperoxybenzoic acid and circle the oxygen atom that forms the epoxide of cholesterol.
2. How could the Beilstein test be used to show that 3-chlorobenzoic acid has not eluted from the chromatography column with the cholesterol? How is this test run and what is observed? Refer to any Organic Lab text (CRC Tutorial Room has Williamson & Durst and Gokel).