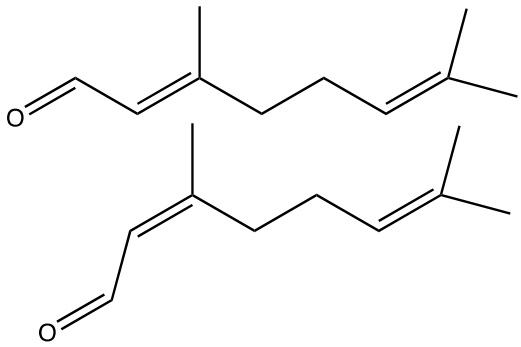
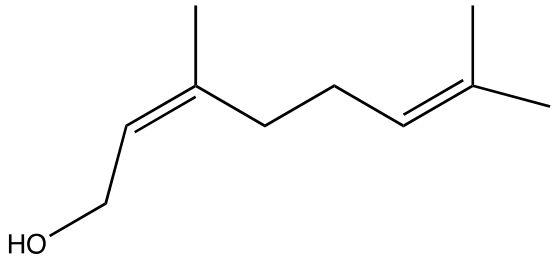
Synthesis of the Terpene Limonene: An Interesting Acid Catalyzed Carbocation Rearrangement of the Terpene, Nerol. Application of GCMS to Structure Determination

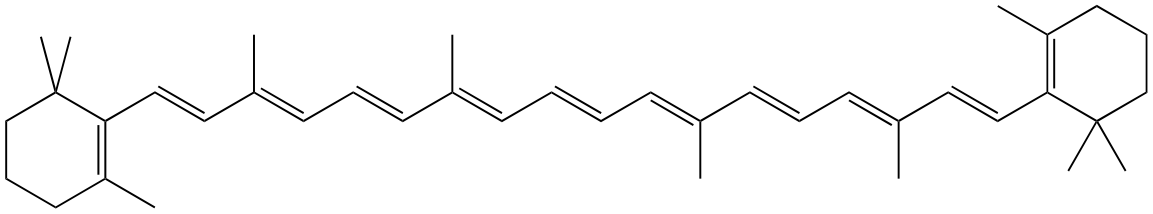
In preparation for this lab read section 17.6 of your current lecture textbook, Loudon and Parise and study the background and procedure provided in this document The referenced section in Loudon and Parise describes a class of compounds known as terpenes, their utility and how they are biosynthesized in living systems. Terpenes are a class of important natural products. Compounds that are derived from terpenes are called terpenoids. You may already be familiar with the names of some terpenes and terpenoids such as beta-carotene, cholesterol, estradiol, estrone, testosterone. Less likely is that you may have heard of the geometric isomers, neral and geranial that comprise citral, the major constituents of lemon grass oil. This oil is used in cooking and to scent as lemon scented cleaning products. Have you every squeezed the skin of an orange? You should try it today! If you do, you will observe a liquid spray out of the pores of the orange skin. You will also experience the scent of limonene which is a major component of the spray and is a terpene. In a somewhat related manner to its synthesis in nature, limonene and a few related isomers can be easily synthesized in the laboratory from a close terpene relative of neral, called nerol. It is the reduced form of the aldehyde neral shown below.



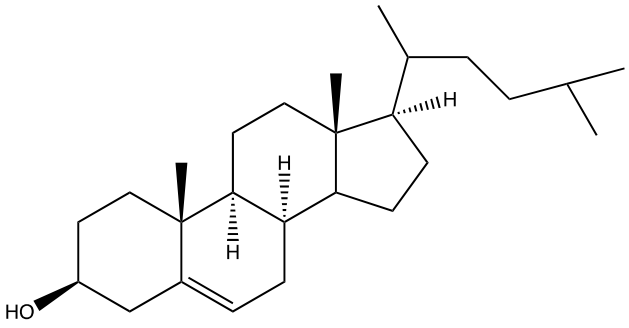
citral: geranial and neral



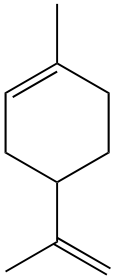
nerol



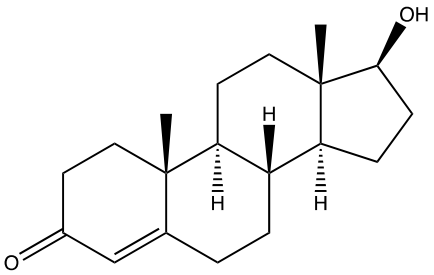
beta-carotene



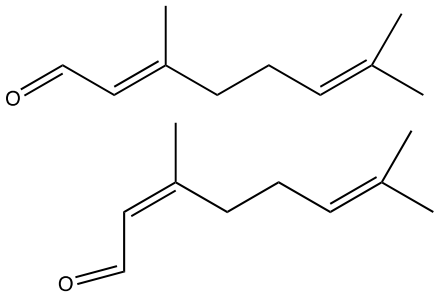
cholesterol



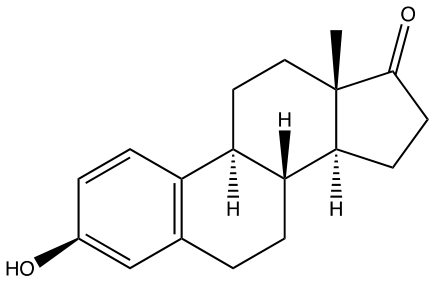
limonene



testosterone



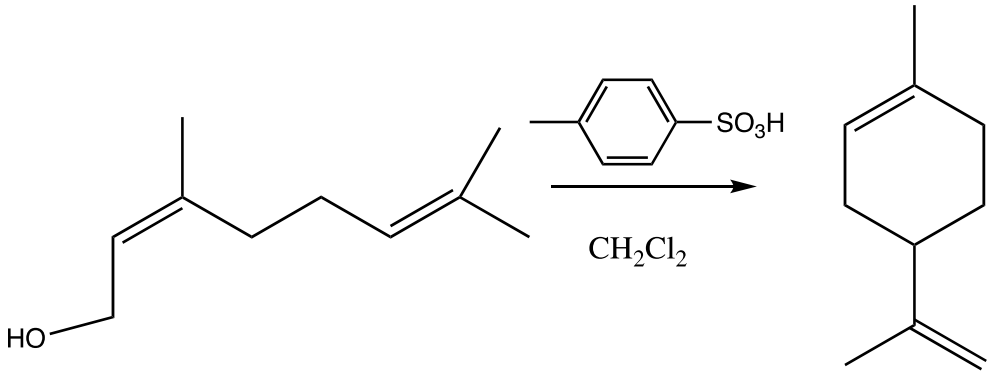
citral: geranial and neral



estrone

Well Known Terpenes and Terpenoids

When nerol is dissolved in an inert solvent like dichloromethane and treated with a catalytic quantity of p-toluenesulfonic acid, nerol protonates and dehydrates forming a resonance stabilized carbocation. This cation cyclizes to form limonene and other closely related compounds as bi-products. It will be your responsibility in this laboratory to prepare to carry out the procedure outlined below. In lab you will complete the procedure and isolate the crude mixture of products formed. The crude mixture will be analyzed for molecular masses as well as structure using GCMS. By now, you have submitted unknowns for GCMS analysis and can do so again this week, though you will go further in your analysis and hopefully your understanding of the application and utility of GCMS. It will also be your responsibility to complete the following worksheet (one per student). This worksheet with comprise your write-up.



nerol limonene

Acid catalyzed conversion of Nerol to Limonene

The Synthesis of Limonene from Nerol.

Procedure:

1. Obtain a clean, 2 dram vial (black top vial) from your instructor or TA.
2. Measure out 0.3 grams of nerol into the vial. It is acceptable to weigh the liquid reagent into the vial. Be careful and do not waste material. What do you do if you put too much in? You don't start over and you don't put it back in the reagent bottle. What is your salvage plan.
3. Add 3 mL of dichloromethane and 0.05 gram of p-toluene sulfonic acid to the vial. It is prudent to weigh the p-toluenesulfonic acid onto piece of weighing paper before adding it.
   As noted earlier, p-toluenesulfonic acid is very corrosive and is extremely hygroscopic. Please follow safety instructions in the associated safety section on the web and put the lid back on the reagent bottle.

4. Add a micro stir bar (you will be given one) and allow the reaction to stir for forty-five minutes to an hour.
5. The reaction will not be complete at this time. This is an acceptable situation as we will be interested in the product mixture, not a complete conversion. Take up approximately half your reaction in a pipet and add it to a separate vial. Add about 1 mL of saturated sodium bicarbonate to the first vial. Cap the second vial (keeping it just in case the reaction has not produced enough product for analysis) and stir the first, quenched vial for five minutes. The reaction may bubble. Why? It is possible you will carry out this neutralization step two times. Vent the lid on the vial approximately every thirty seconds.

6. Allow the vial to settle and pipet out the lower layer (tip the vial and dip the pre-squeezed pipet into the lower layer . Dip the pipet into the deepest part of the lower layer and carefully remove most of the lower layer). Some people prefer to take off the upper layer. Do what you feel most comfortable with. You do not need to get it all, you just need enough for analysis.

7. Add the organic layer (hint: it is predominantly dichloromethane) into a small vial containing about a millimeter of anhydrous sodium sulfate. Make sure you recap the sodium sulfate bottle. Please use a light hand with the sodium sulfate. How do you know when a reaction is dry? The major indicator is that the solution looks clear – that is an adequate criterion in this micro-reaction.

8. Make sure you keep your vials straight – keep them labelled with the tape that is available in the lab.

9. Take about 8-10 drops of the dried liquid and add it to a provided GCMS vial and dilute the vial to about 2 mL with dichloromethane. Some CCMS vials have graduation. Generally, filling the vile about three quarters is adequate.

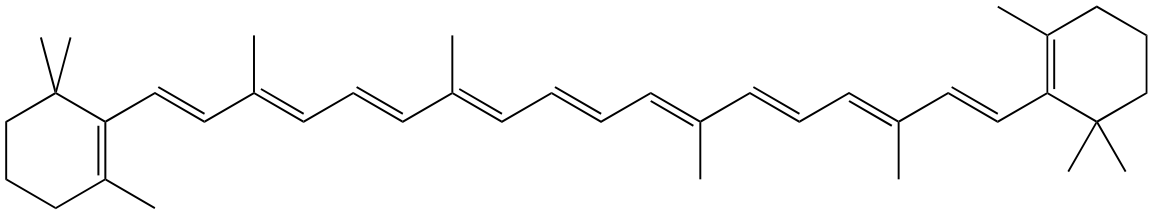
10. Tightly cap you vial and label your vial with your initials and your lab day and give it to your TA or instructor to run. Do not put tape on the GCMS vial – use a sharpie. Generally tape does not belong on containers that will be used in a robotic instrument. Why?

11. Submit your sample to your TA or instructor for analysis.

Worksheet – Completion of this Worksheet will Comprise your Write-up

While you are waiting for you GCMS, try to complete the following worksheet.

1. Consider the following terpene or terpenoid compound. Find all the isoprene units in this terpene. Circle them. An isoprene unit is essentially a 2-methylbutane unit. The double bond position does not matter in choosing the unit. (8 points)



beta-carotene

2. What is the significance of terpenes in biology and biochemistry. Read the assigned section in the text book if you have not already (8 points)

3. Look at the attached typical (this is a practice GCMS – it does not relate to your reaction) GCMS. Answer the following questions about the information it contains.

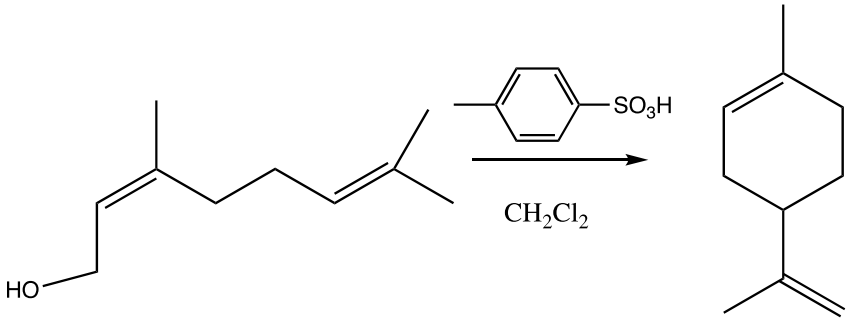
    a. Given the fact that it is first a Gas Chromatograph, how many compounds are in the reaction mixture? (8 points)

    b. For which peak is the GCMS data given? (6 points)

    c. Given the bit of introduction you have had to GCMS, can you determine the molecular weight of the compound emphasized in the GCMS trace. (8 points)

d. Looking at the practice Mass Spectrum can you loosely define a fragmentation pathway occurring in the mass spectrometer. A mechanism is not needed, just define a fragment that is coming off the mass ion in the mass spectrometer. (12 points)

4. Consider the following desired transformation related to your laboratory. Can you write a reasonable carbocation mechanism to explain its formation? (12 points)



5. When you receive your GCMS data (either at the end of lab or later in the week), consider the following questions.
   a. How many significant compounds do you have in your mixture (major GC peaks at 5.5 minutes or less).(4 points)

   b. Which compounds in the first 5.5 minutes have the same molecular weight as the reactant? (6 points)

   c. Which seem related in terms of molecular weight to limonene and appear to be isomers? 8 points

d. Using the library printout given with your GCMS data and your extensive knowledge of nomenclature, assign the major peaks in this spectrum (the four or five biggest peaks in the spectrum in the first five or six minutes of the GC). (12 points)

\

e. Do you have any significant products in addition to limonene. If you do, can you write mechanisms for their formation? Refer to your mechanism for the formation of limonene. (12 points)

6. For limonene or one of its closely related bi-products, can you loosely define one major fragmentation process that is occurring. A mechanism is not needed, just define a fragment that is coming off the mass ion in the mass spectrometer. (12 points)

7. It was stated in the procedure, that the reaction would not finish in the time given and under the given reaction conditions.
What evidence do you have as to the progress of the reaction? What ideas do you have to improve the yield of the reaction? (12 points)

8. Of the isomers you isolated, which alkene would you predict to be the most stable based on what you have learned in lecture? (6 points)