Chemistry 2312 September 17, 2024

Honors Organic Chemistry Laboratory T. R. Hoye

Experiment 2

Ozonolysis and Hydrogenation of Naturally Occurring Alkenes

Report Due Saturday October 5, 2024

**** You can perform the two reactions in this experiment in *either order*. ****

Interim Benchmark NMR for Report 2: Due Sat., September 28, 2024 either compound **3** or **5-cis**

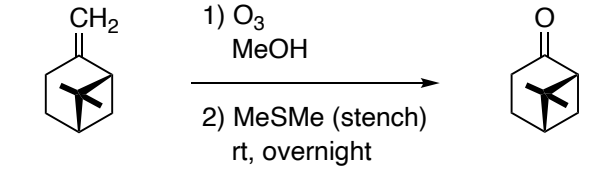
For background, you should read the appropriate sections in your textbook dealing with ozonolysis of alkenes and hydrogenation of alkenes.

Read in Mohrig Chapter 8. Computational Chemistry

Chapter 12. Boiling Points and Distillation

(Note to self: I should hand out Spectroscopies Worksheet #1 prior to the due date for Experiment 2.)

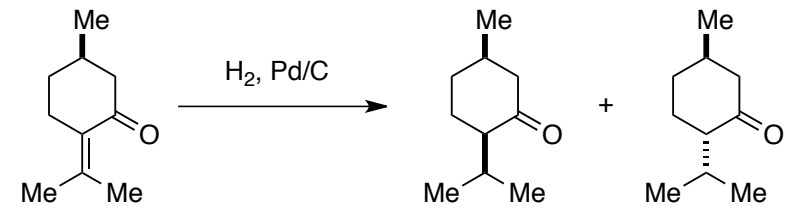
a. *Ozonolysis: Nopinone (3) from β-Pinene (2)*



(+)-β-Pinene
2

Nopinone
3

b. *Hydrogenation: Isomenthone (5-cis) and Menthone (5-trans) from (R)-(+)-Pulegone (4)*



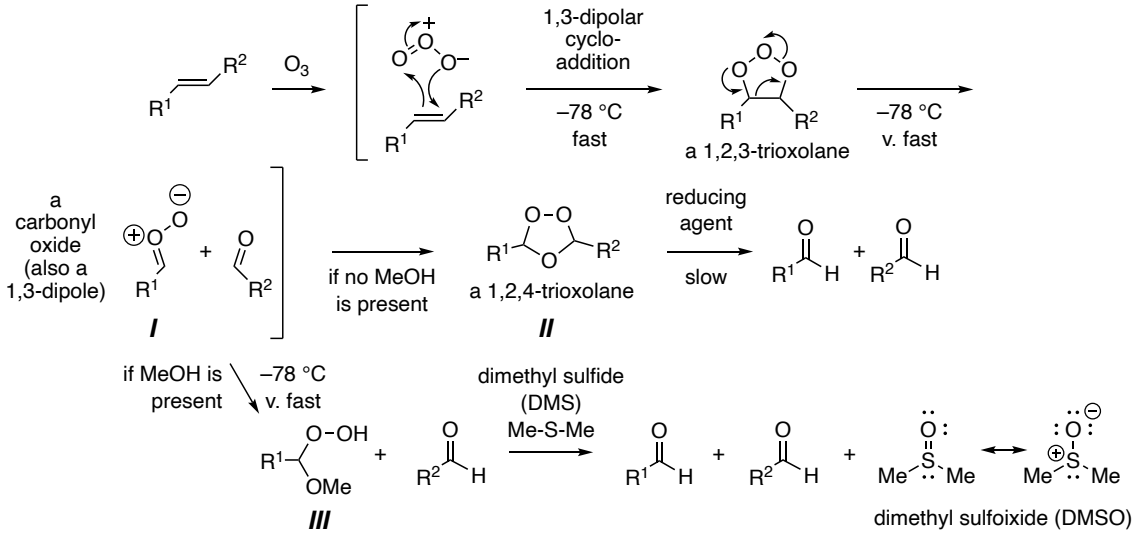
(*R*)-(+)-Pulegone
4

5-cis

5-trans

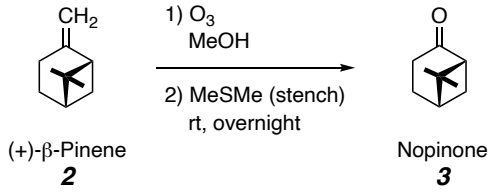
a. Ozonolysis of β-Pinene:

The transformation of alkenes to carbonyl compounds is a widely used reaction in organic synthesis. There are several methods of oxidizing alkenes. The products of these oxidations include aldehyde, ketones, diols, epoxides, and alcohols. The oxidative cleavage of alkenes to aldehydes and/or ketones can be accomplished by several reagents. One of the classical reagents to oxidatively cleave an alkene is ozone, which is generated from molecular oxygen. The ozonide products resulting from net 1,3-dipolar addition of ozone to the alkene are rarely isolated. Instead, they are usually reduced *in situ* to a pair of aldehydes and/or ketones, depending upon the degree and location of substitution of the original alkene.



Cyclic alkenes also can be ozonolyzed to provide a difunctional product. The oxidative cleavage of alkenes to aldehyde or ketone products provides a more highly functionalized product than that of the original alkene. Ozonolysis is also used in the degradative determination of unsaturated unknowns. The two smaller fragments can often be more easily identified by spectroscopic methods. Knowing the structure of the aldehyde(s) or ketone(s) can allow one to deduce the structure of the precursor alkene.

Experimental Guidelines for the Ozonolysis Reaction:



Ozone is typically generated from molecular oxygen by electrical discharge--you may have smelled it following a lighting strike at the beginning of a storm or while using an electrical device that arcs. The ozone generator in the lab will produce a flow of ozone that you will use to oxidatively cleave β-pinene (**2**) to nopinone (**3**). You will use a gas-drying tower as the reaction vessel. Ask one of the TAs to get enough dry ice for the large Dewar flask cooling bath, which will have acetone as the liquid. I recommend that you convert approximately 6 g (accurately weighed) of β-pinene to nopinone in this reaction. The **reaction solvent will be methanol**. Solutions of ozone in a polar protic solvent like methanol (or methanol-containing mixtures) are blue. However, the ozone will react extremely rapidly with alkenes for as long as some amount of alkene is still present. Thus, when the supply of alkene is exhausted (i.e., the reaction has reached 100% conversion), a royal blue color, resulting from a methanol-ozone complex, should be observable. More importantly, the methanol traps the intermediate carbonyl oxide dipole

(intermediate **I** in the mechanism scheme above) to give the hydroperoxide **III**. This hydroperoxide is much more easily (i.e., quickly) and reliably reduced to the two carbonyl products than is the cyclic peroxide **II**, which is formed if methanol is not used as the reaction co-solvent.

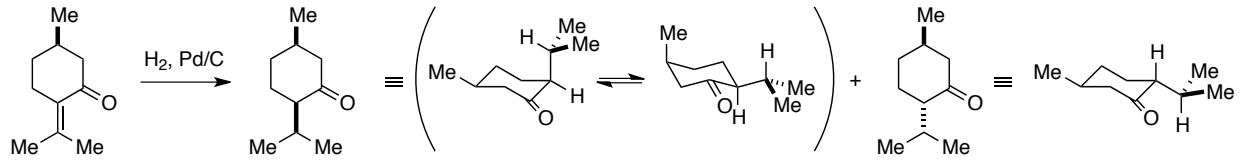
Perform the reaction using an initial concentration of pinene of **0.5 M** in methanol. Set up the ozonolysis in the fume hood immediately to the left of the ozone generator. Attach the tygon tubing from the ozone generator to the glass bubbler inlet tube on your glass reactor. Cool the reaction vessel in a dry-ice/acetone cold bath (which will be at ca. -78 °C). Follow the directions posted on the generator and allow the reaction to proceed to completion. Remember, the formation of the blue methanol-ozone complex should indicate when the reaction is complete. Turn off the ozone generator but allow oxygen to continue to flow through the generator and reaction mixture to sweep the excess O_3 out of the apparatus and your reaction solution. Once the blue color has discharged (ca. 10 min), disconnect the tubing from the reactor (to prevent suck-back of the reaction mixture into the tubing), turn off the oxygen flow at the main tank valve, and proceed with the reduction of the ozonide intermediate. Remove the reaction vessel from the cold bath, wipe any acetone/dry ice from the tower with paper towels (**caution: dry ice/acetone can cause frostbite if it meets skin for more than a few seconds and the portion of the vessel that was emerged will be very cold**), and allow the vessel and contents to warm back to near room temperature. Pour the reaction mixture into a ≥250 mL round bottom flask; aid the quantitative transfer by rinsing with a few more milliliters of fresh MeOH. Check this solution for the presence of peroxides (see several sentences below). Add 1.5 equivalents (**show your calculation to a TA to ensure a proper amount is being used**) of dimethyl sulfide (CH_3SCH_3, DMS, **Stench! KEEP IN HOOD**) to the transferred reaction mixture to reduce the intermediate peroxide. Swirl the contents to achieve a homogeneous solution and allow this solution to stand, inside the fume hood, at room temperature for overnight (or longer). Lightly cap the flask with a yellow plastic cap-plug. **Because peroxides are potentially explosive, it is critical that all traces of peroxide be reduced by the DMS before proceeding with the vacuum distillation.** The consumption of peroxides can be monitored with the use of starch-iodide (SI) paper—a sensitive technique for visualizing the presence of trace levels of peroxide. Place a single drop of your reaction mixture, while it is incubating with DMS, onto a strip of dry SI paper. Allow the solvent to dry/evaporate (10–30 sec). *Then* add a drop of water to the same area of the SI paper. The presence of peroxide will be indicated by a dark blue-black-purple-brown color. Perform an initial control analysis *before* adding any DMS so that you know what to expect for a positive indication. **Show the result of your negative starch iodide test, indicating the consumption of all peroxide, to a TA before proceeding beyond this point.** Concentrate the reaction mixture **on the rotary evaporator located inside the east hood** in order to isolate the crude product. This will also remove the excess of volatile DMS (bp 37–38 °C) and doing it in the hood prevents the DMS vapors from escaping into the lab atmosphere. Redissolve the non-volatile material in 20–30 mL of hexanes and wash twice with water. This will remove the DMSO ($Me_2S{=}O$) byproduct, a very polar and highly water-soluble compound. Dry the organic layer ($MgSO_4$), filter, and concentrate to provide the crude product. Record the mass. Finally, distill the product under reduced pressure using the vacuum distillation set-up located inside the hood in the southeast corner of the lab. (See the posted separate 'handout' on the protocol for vacuum distillation.) Record the temperature range of the distillate and the pressure in units of torr (= mmHg). **Carry out the vacuum distillation with the hood sash doors closed.** Obtain yield (mass) and spectroscopic data on this purified (i.e., distilled) product.

Handle all glassware that has had any contact with DMS in the fume hood. Rinse *any glass surface* that has had such contact with a dilute solution of bleach (NaOCl)*, which we will keep in the hood beside the rotary evaporator in the east hood, prior to removing the glass item from the hood for additional cleaning. *Jack: Please prepare a bleach bath (dilute the bleach 1:5 with additional water) in a wide plastic container, label it properly, and place it the (east) hood between the rotary evaporator and the tlc staining station.

b. Hydrogenation of Pulegone:

The addition of hydrogen across carbon-carbon π-bonds results in reduction of the alkene (or alkyne) to the corresponding alkane ("saturation" of the carbon atoms to bear their maximum number of hydrogen atoms). This reaction requires a catalyst because the uncatalyzed reaction is too slow to occur at any practical rate. Dozens of hydrogenation catalysts, both homogeneous (soluble) and heterogeneous (insoluble, solid suspensions) are known. Perhaps the most used catalyst consists of finely divided palladium metal deposited on carbon powder (Pd°/C). This provides a large surface area, upon which reaction takes place when both hydrogen gas and the substrate alkene are adsorbed on the surface of the palladium metal particles.

Experimental Guidelines for the Hydrogenation Reaction:



(*R*)-(+)-Pulegone

4 ***5-cis*** ***5-trans***

Plan to hydrogenate ~250 mg of pulegone (**4**) in a culture tube or round-bottom flask having a volume of 10–25 mL. Use ~2–4 mL of 95% ethanol or ethyl acetate as the reaction solvent and (a measured amount) of ~25 mg of 10% Pd°/C. *Katharine: has prepared small, capped vials, each containing ca. 25 mg of 10% Pd°/C and labeled as such and placed one on each of your benches. Since a mixture of palladium on carbon powder, air (more specifically, oxygen), and flammable organic solvent vapor can spontaneously ignite, the order of addition of reaction components is important. We will add things in the following order: (i) first, place the dry catalyst powder in the bottom of the reaction vessel (e.g., a culture tube outfitted with a magnetic stir-bar and capped with a rubber septum), (ii) flush the air from the headspace of the vessel with a *gentle* stream of nitrogen (house N_2 lines are in each hood; always close the valve after using—the N_2 gas, a somewhat expensive commodity, comes from boil-off from a liquid nitrogen storage tank located in the back of Smith Hall), (iii) add a small portion of the reaction solvent by syringe (enough to cover the catalyst with a layer of solvent). Then add a solution of your pulegone in the remaining volume of reaction solvent. Evacuate the headspace with the house vacuum line and admit hydrogen gas (via a balloon) into the reaction flask. *Please ask the TA to assist you in filling your balloon with hydrogen.* Be prepared to add the hydrogen gas to the headspace of your reaction vessel quite soon after the pulegone and solvent have been placed over the Pd/C. Pulegone will be slowly converted into thymol (a phenol derivative) by Pd/C in the absence of H_2. *It is important that you do not add hydrogen gas to the dry Pd/C catalyst, so review the order of operations stated above immediately before you set up this experiment.* The hydrogenation reaction should occur readily at room temperature. However, you must still monitor the reaction progress by tlc of a small aliquot. The product and starting material will have very similar R_f values, but they will show differential detectability behaviors under UV vs. staining visualization. This is because the products, aliphatic ketones, do not strongly absorb UV light. When all the starting alkene is consumed, remove the catalyst by filtering the reaction slurry through a small pipette column of Celite®, packed as a slurry in the same solvent in which the reaction was run. [Discard the packing material of your Celite® column, which now contains the residual or spent Pd/C catalyst, in a solid waste receptacle dedicated for collection of palladium-containing waste (and labeled as such-).

Separate the *cis*- and *trans*-isomers of the hydrogenated products **5** by medium pressure liquid column chromatography (MPLC). This separation is achievable by MPLC (and can even be accomplished by flash chromatography, although the latter is challenging). Carefully choose an elution solvent in which the tlc R_f is ~0.2. Characterize your sample of each pure diastereomer by GC-MS, IR, and NMR spectroscopy. Report the yield of each isolated, pure diastereomer.

Once each pure isomer is in hand, **isomerize** a small portion (~10 mg) of *each* separate isomer to afford the same equilibrium mixture of the two by treating separate methanol solutions (ca. 1 mL) **of each isomer** with Amberlyst 15, a cross-linked, resin-based, Brønsted acid catalyst. Use ca. 25 mg of the Amberlyst; the beads tend to be fairly staticky, so weighing them directly into your otherwise empty reaction vessel is a good option. The reaction vessel should be your smallest volume culture tube, fitted with a screw cap; magnetically stir the suspension (Amberlyst, a polymeric resin, is insoluble). Monitor and quantify the equilibration by gc by taking a small quantity of the reaction solution (avoid withdrawing any of the resin) and diluting it further with EtOAc to prepare a GCMS sample. If the suspension has become pulverized and a crystal-clear aliquot cannot be removed, we will need to filter the aliquot first through Celite to prevent fine particles from clogging the needled on the GC autosampler (a big No-No). Keep in mind that you are still looking to achieve an ca. 1 mg per 10-100 mL final concentration of that sample as you perform the GC-MS analysis. *You do not need to reisolate the two components after the equilibration*, so you do not need to be concerned about quantitative recovery at all; simply determine the ratio of the two isomers from the integrations of the gc peaks. Both of the menthone and isomenthone diastereomers should equilibrate to essentially the same equilibrium ratio of the two, governed by their difference in free energy ($\Delta G°$).

In previous years, we performed the calculations below. Because we are short-staffed on TAs this year and the logistics of implementing these computations are quite burdensome, we will not have you do these calculations this year. I later will provide guidance on how you can use the results from one of the TA's computations into your Report #2.

Update on 10-1-24

I will have you work with our (now) computed DFT energies of the conformations of the trans- and cis-menthone isomers, but I will need to provide specific guidance later on what that addendum will entail. I will also give a lecture so that you can learn about the process of determining the energies of multi-conformations of diastereomers.

For the report you turn in on Sat. 10-4-24

• Write a short description of procedures you used for each of the equilibration experiments of the pure menthone diastereomers.

• Turn in a GCMS chromatogram for **each** of the two experiments. Indicate which is which. Report the integration ratios of the peak areas for each of the two chromatograms.

Molecular Mechanics Computations to Assess Relative Energies of 5-cis and 5-trans

Using the MMFF force field in the program MacroModel [accessed via Maestro and the Minnesota Supercomputer Institute (MSI)], carry out a multi-conformational search (a Monte Carlo search) and compute the relative steric energies of **5-cis** and **5-trans**. Calculate an equilibrium constant from these relative energies and compare the calculated value to the experimentally observed value. Consult the PDF files I have placed on the website for how to create your own spreadsheet in which to tabulate and Boltzmann weight the energies of each of the conformations you locate in the computational study for **5-**

cis and **5-trans** and to then deduce the computed free energy difference (and associated equilibrium value).

Add a separate page to your report where, in a single paragraph of text, you summarize the results of your calculation. Include the relative energy of the cis- and the trans-isomers. Indicate what MM forcefield you used and what solvation model you applied. Also include as an attachment:

(***i***) the Excel spreadsheet where you have performed the calculations of the Boltzmann averaged energies of the cis- and the trans-isomers.

(***ii***) a GC chromatogram of one of your equilibrated mixtures of the two diastereomers.

Include answers/discussion to the following at the end of your lab report for Experiment 2:

1) If the total gas flow of ozone in oxygen is 10 mL min^{-1} and that gas stream contains 1.5 vol% of ozone, how long should it take to consume 30 mmol of β-pinene (**2**)? Assume that the apparatus allows for 100% efficiency; that is, that every molecule of ozone introduced into the solution inside the reaction flask consumes one molecule of the reactant alkene. Show your calculation.

2) Provide a mechanism for the ozonolytic cleavage of (*E*)- 1-methylcyclopentene α-pinene (this is an isomer of β-pinene—look up its structure) by ozone in CH_2Cl_2 in the absence of methanol, followed by a reductive workup of the ozonide using tributylphosphine ($^nBu_3P$, 1 equiv). What byproduct is formed from the tributylphosphine that is oxidized? What product is formed from the alkene substrate?

3) If you were to record the ^1H NMR spectrum of a sample of your crude nopinone product obtained simply by removing (rotary evaporator) most of the volatile components of your reaction mixture, you would observe singlets at δ ~3.4 and ~2.5 ppm. These are from two different compounds and their ratio of intensities would be a function of how long you left the sample on the rotary evaporator. The resonance at 3.4 ppm would disappear upon extended time on the evaporator. What two compounds are responsible for each of these two singlets?

4) If 3.0 g of cyclooctene is reduced to cyclooctane by the addition of hydrogen gas, what volume of H_2 would be consumed? Assume that hydrogen is an ideal gas, that the lab temperature is 25 °C, and that the atmospheric pressure is 760 mm of Hg. Show your calculation.

5) If this hydrogenation reaction were performed in Denver, Colorado , would the volume of hydrogen gas uptake be greater or less than you calculated for question 4? By approximately what amount: 1% greater or less, 10% greater or less, or 100% greater or less? (recall that pV = nRT – always has and always will)

6) If the substrate for an ozonolysis is carried out in a reaction solvent that does not give rise to blue color when excess ozone is present, this is not a serious problem. However, it is more difficult to determine when the reaction is complete, since excess ozone does not give a dark, blue-colored solution in pure methylene chloride. A trick to circumvent this problem is to attach a piece *latex rubber* tubing to the vent of the gas tower you use as the reaction vessel. At the end of the reaction the tubing quickly crumbles into small pieces. Explain what is happening. [Hint: look up the structure of the polymer that composes natural latex rubber.]

7) Suggest a mechanism by which pure **5-cis** can be isomerized to the equilibrium mixture with **5-trans** in the presence of a *basic* catalyst (e.g., NaOH). [You may want to (re)read in your organic chemistry textbook about the mechanism for "keto-enol tautomerization".] What would be the outcome if you were to perform this isomerization in a solution of $NaOD/D_2O$?

8) If molecule A is 2.7 kcal/mol more stable (*i.e.*, has a lower free energy) than its isomer B and you effect interconversion of A to B, what will be the equilibrium ratio of A:B at room temperature? (recall that $\Delta G° = -RTlnK_{eq}$).