

Isolation and Characterization of Essential Oils

The first two laboratory sessions will concern the isolation and characterization of the terpenes present in the essential oils of cumin and caraway seeds. The first week will be spent isolating either cumin seed oil or caraway seed oil, and the second week will entail spectroscopic analysis of the oils, pure terpenes present in these oils, and other terpenes.

Week I--Isolation of the Essential Oils of Cumin or Caraway Seeds

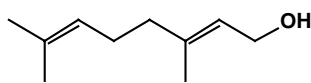
Pre-lab

Reading: Read pp 54-60 in Mohrig, et. al. and review the techniques of steam distillation and extraction.

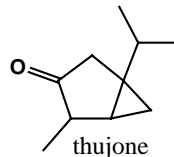
Notebook: Make sure your notebook has an up-to-date table of contents and numbered pages. Write an experiment title and purpose, followed by *brief* descriptions of the purpose and general procedure to be used. Draw the structures of the major component of caraway seed oil, (S)-(+)-carvone, and the major component of cumin seed, cuminaldehyde (4-isopropylbenzaldehyde), in your notebook, along with their relevant physical properties (boiling point, density, optical rotation). Also include the physical properties of water and dichloromethane. As the experiment progresses record pertinent observations in your notebook.

Questions: Answer the following questions on a separate sheet of paper and turn them in to your TA upon entering the laboratory.

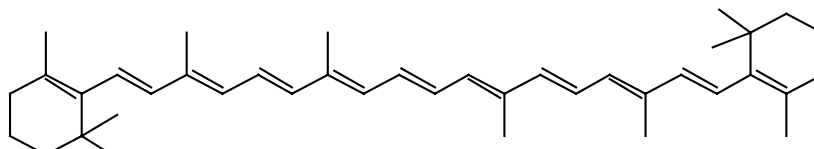
1. Show the isoprene units in each of the structures listed below.



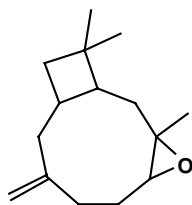
geraniol



thujone



β -carotene



caryophyllene oxide

2. Why is the distillation temperature of the system constant?
3. Will dichloromethane constitute the top or the bottom layer in your separatory funnel?
4. How will you know when you have evaporated all of the dichloromethane away from your essential oil?

Experiment

You will be assigned either cumin seed or caraway seed to extract. Once you know which substance you are working with, follow the procedure in Mohrig, et. al., described on pp 59-60, **with the following exceptions:** *You will not be making a semi-carbazone derivative of carvone. You will not use gas chromatography or polarimetry to analyze your products, but rather IR and NMR spectroscopy (described below).* Save your product in your drawer, **tightly stoppered**, for the second week.

Week 2--Spectroscopy of Terpenes

Although the essential oils that you isolate will contain one major component, either carvone or cuminaldehyde, there will also be other terpenes present in your product. The purpose of the second week's experiment is to evaluate the purity and identify the components of the essential oils of cumin and caraway seeds using IR, ^1H NMR, and ^{13}C NMR spectroscopy. In this process, you will assign spectral absorbances to particular structural features of the terpenes under consideration, and you will learn how to use and interpret spectra from a DEPT (Distortionless Enhancement by Polarization Transfer) NMR experiment.

Before reading the rest of this laboratory experiment, review Chapter 13 of Brown and Foote, 2nd Edition, and closely read sections 13.13 and 13.14.

You are already familiar with interpreting IR and ^1H NMR spectra in order to identify the structure of unknown compounds. Sometimes ^1H NMR spectra are too complicated to accurately identify and assign all of the signals--this is particularly true for many natural products and polymers. In this case, investigation of the proton-decoupled ^{13}C NMR ($^{13}\text{C}\{^1\text{H}\}$ NMR) spectrum can yield greater insight into the actual compound structure and purity.

The ^{13}C NMR spectra are simpler than the proton spectra because the complex splitting due to neighboring proton nuclear spins is removed by decoupling. In addition, the coupling between neighboring ^{13}C nuclei is not observed because of the very small chance that two ^{13}C atoms will be neighbors in any given molecule. (Remember, the natural abundance for the ^{13}C isotope is only 1.1%.) In general, one single resonance is observed for each chemically distinct carbon in the molecule. The trend in relative chemical shifts of carbons, measured in ppm relative to TMS (tetramethylsilane), closely matches that observed for proton NMR. For example, aliphatic carbons resonate farthest upfield, carbons attached to polar functional groups resonate farther downfield, aromatic carbons resonate even farther downfield and carbonyl carbons resonate the farthest downfield from TMS.

Because the proton decoupling can affect the intensity of the ^{13}C signals, we do not normally integrate carbon spectra. Some useful information can be gleaned from relative intensities, however. When comparing resonances due to similar carbons (for example, if you compare resonances due to aliphatic CH_3 groups with one another), it is reasonable to interpret two signals that are in a 2:1 ratio as representing a 2:1 ratio of carbon atoms in the molecule. It is not possible to compare intensities of two different types of carbon atom (for example, don't compare CH_3 carbon intensities with CH carbon intensities). Also, quaternary carbon atoms (sp^3 carbons bearing no hydrogens) and other carbons with no hydrogens, such as those contained in a benzene ring and

bearing a substituent, can often be identified because they generally produce the least intense signals in the $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum.

How do you know which signals are due to primary (CH_3), secondary (CH_2), tertiary (CH) or quaternary carbons? The DEPT (distortionless enhancement by polarization transfer) experiment is a useful NMR experiment that provides information on the number of protons attached to the various ^{13}C resonances in a carbon NMR spectrum. In a DEPT experiment, the spins of both ^1H and ^{13}C nuclei are manipulated, through a sequence of carefully timed delays and RF pulses, so as to cause enhancement of the intensities of the ^{13}C signals. In addition, the experiment is designed to manipulate the phase of each ^{13}C signal so as to allow differentiation of the signals according to the number of attached hydrogen atoms. The behavior of the ^{13}C spectrum in three differently configured DEPT experiments is outlined below.

DEPT 45

This experiment provides a ^{13}C spectrum that is missing signals from carbon atoms that have no attached hydrogen atoms (directly bonded hydrogen atoms are necessary for the polarization transfer to occur). The phases of all of the ^{13}C signals in the DEPT 45 experiment are positive, giving rise to normal (+) peaks in the frequency spectrum. The DEPT 45 spectrum thus allows one to identify, through comparison with the normal ^{13}C NMR spectrum, quaternary carbon atoms, alkene and aromatic carbon atoms which do not have attached hydrogen, and non-aldehydic carbonyl groups. Chemical shift information is, of course, used in conjunction with the DEPT data.

DEPT 90

This experiment provides a ^{13}C spectrum that contains only the signals from carbon atoms that are bonded to a single hydrogen atom (methine, aromatic and olefinic C-H, aldehyde). The signals have the normal (+) phase, and thus give rise to normal peaks in the frequency spectrum. Signals from CH_2 and CH_3 groups do not appear (the signals are nulled). It should be noted that careful adjustment of both ^{13}C and ^1H pulse lengths is necessary for exact nulling of the methylene and methyl signals. If either of these parameters is improperly set, small residual signals from the CH_2 and CH_3 carbon atoms will appear in the spectra.

DEPT 135

This experiment provides a ^{13}C spectrum that contains signals, as does the DEPT 45 spectrum, from all carbon atoms that are bonded to any number of hydrogen atoms. In the DEPT 135 experiment, however, the phases of the signals from CH_2 carbons are inverted, and thus give rise to negative peaks in the frequency spectrum.

When you view the DEPT experiment data, three separate data sets, one each for 45, 90, and 135 will be plotted. By evaluating the resonances in each data set, whether they are positive, negative, or null, you can identify the number of protons attached to each carbon. This information will then provide evidence for the most likely structure(s) that can be assigned to the compound in question.

Prelab

Notebook: Include a title and statement of purpose, and a *brief* description of the procedure (a list of steps you will perform is sufficient). It might be useful to reference an explanation of the spectroscopic techniques you will employ.

Procedure

There are four major tasks to accomplish during this laboratory:

1. Analyze the DEPT NMR spectra of five terpenes (handout in the laboratory), so that you can assign the spectra to particular terpene structures.
2. Combine your essential oil with that of two other lab students who isolated the same material, and prepare a sample for obtaining a ^{13}C NMR spectrum in CDCl_3 .
3. Obtain a ^{13}C NMR spectrum of your product on the Varian 300 MHz NMR instrument in NHS 208, along with your two partners, and make five copies to share with your benchmates.
4. Download the ^1H NMR spectra of carvone and cuminaldehyde using the PC-based program, Mest-ReC.

At the end of the laboratory period, you should have the following information:

1. IR and ^{13}C NMR spectra of cumin seed oil and caraway seed oil.
2. IR and ^1H NMR spectra of carvone and cuminaldehyde.
3. DEPT and ^{13}C NMR spectra of the five terpenes drawn below (including carvone and cuminaldehyde) (handout).

Detailed Instructions:

DEPT Spectra

The following five terpene structures all have the same number of carbon atoms. If you inspect the structures carefully, you will notice that they have different numbers of primary (three attached Hs), secondary (two attached Hs), tertiary (one attached H) and quaternary carbons (no attached Hs), however. Therefore, the DEPT spectra should enable you to distinguish these compounds from one another. Obtain copies of DEPT and ^{13}C NMR spectra labeled "Terpenes A-E" upon entering the laboratory and begin analyzing the number of each type of carbon present in each spectrum, according to the explanation of each DEPT experiment written above. After matching the terpene structure with its respective ^{13}C NMR spectrum, label the ^{13}C resonances according to the letters used to label the individual carbon atoms in the structure.

Terpene structures:

^{13}C NMR Spectrum

1. Preparation of the ^{13}C NMR Sample

You will be running three samples: the oil which you isolated by steam distillation, pure carvone, and pure cuminaldehyde. DEPT spectra will be obtained for the latter two. All samples will be dissolved in deuteriochloroform (CDCl_3). This solvent will serve both as the source for the deuterium lock signal and as an internal reference ($\delta^{13}\text{C} = 77.0$ ppm). Your instructors will show you how to measure appropriate volumes of both solvent and solute. The volume of solvent is critical - too much solvent not only dilutes the ^{13}C spins, but also greatly increases the difficulty of shimming the sample. It is imperative that the sample be dry and free of solid impurities. Should either water or insoluble material be present, it must be removed before running the spectrum. Techniques for both drying and filtering of the samples can be demonstrated by your instructors as necessary.

2. Operation of the Varian NMR Spectrometer

Notes: • Be careful to wait for the instrument to respond to the command you have given before giving the next command. Sometimes the computer can be slow

and try your patience. If you aren't patient, the computer may hang up or crash.

- Throughout this document commands that you type in are denoted in **bold courier font** and the symbol ↵ indicates a return.
- When typing commands remember that UNIX is "case sensitive", meaning that a capital letter is not equivalent to a lower case letter.
- The left mouse button is the normal operator, except in a few cases such as increasing lock or shim values, changing from axial z to z0, and manipulating the spectrum. During some operations you will see three yellow boxes lined up at the bottom of spectrum window. The codes in these three boxes indicate the functions of the left, middle, and right mouse buttons.
- In the VNMR program, to move a window to the "front" simply place the cursor on one of the edges of the window (the arrow cursor becomes an open circle on the very edge of a window) and click the left mouse button.
- Most of the commands you need for routine operation can be performed by clicking on a series of "buttons" in the menu bar above the spectrum window. However, all of these commands can also be entered directly by typing the command code into the cursor window above the menu bar. Sometimes, a particular operation can only be performed by typing in the command. If you are looking to do something different, check the "Command and Parameter Reference" book located by the instrument--chances are, you will find the command you are looking for. Also, it is possible to string commands together on one line, separated by spaces. Type return at the end of the line to execute your commands.
- Click on MAIN MENU to get back to the highest level of "buttons" on the menu bar.

1. Log in to the computer and find your lab section subdirectory within the terpene directory.

When you approach the NMR computer, the screen may be blank. If so, you can turn on the screen by pressing the return key or moving the mouse.

- A. Login as **orgoII** and enter the password (fall1998) at the prompt. On the Sun, this will automatically open Solaris, and dump you into the VNMR operating system. What you will see is a series of windows open. The upper left-hand window will have two rows of "buttons" (the menu bar) along the bottom.
- B. UNIX files and directories are specified by paths in a way similar to DOS files and directories (actually DOS was patterned after UNIX). For instance, the file "junk" may be nested inside a number of directories and its complete pathname might be: /home/orgoII/gretchen/junk, which indicates that junk resides in the subdirectory gretchen, which is in turn a subdirectory of orgoII, etc. All of the user accounts are found in the /home directory. You can move up and down in the directory system in the VNMR program by clicking on the FILE "button". To move down a directory click on the directory of interest and click on the SET DIRECTORY and then CHANGE buttons. To move up a directory click on the PARENT button. The UNIX command pwd is useful to determine where you are (just type it in followed by return). Change into the terpene subdirectory and then the subdirectory appropriate for your lab section (Monday, Tuesday, Wednesday, Thursday)

and check your pathname by typing `pwd` . Your pathname should now read `/home/orgoII/terpene/Monday` (for someone in the Monday section).

As a member of the `nmr` group (a UNIX distinction) all `nmr` user accounts have permission to access and read anyone else's files; however you cannot alter other user's files (of course, this isn't true of the files of other members of your user account, so be careful). You can call up other user's files, save them in your directory and alter them as you like, but you cannot alter the original file. To look at other user's files simply move through the directories as discussed above.

2. Lock and shim your sample.

The sample must be inserted into the magnet probe, the deuterium lock signal located and optimized, and the local field around your sample adjusted to make it as homogeneous as possible (the latter process is called 'shimming', within the context of NMR jargon). Your instructors will show you how to do this after insertion of your sample.

3. Set-up the experiment and obtain spectral data.

Note: Main Menu is the highest level among the menu boxes.

The NMR spectrometer must be set to the proper frequency for ^{13}C and appropriate parameters for ^{13}C signal acquisition must be entered. The parameters are contained in a table within the computer, and are loaded by clicking on the 'setup' button, followed by clicking on the appropriate combination of nucleus and solvent ($^{13}\text{C}/\text{CDCl}_3$ for our measurements).

- A. SET UP THE EXPERIMENT that you want to do with the left mouse button, by clicking on SETUP and choosing appropriate buttons in the command bar above the spectrum window ($^{13}\text{C}/\text{CDCl}_3$ for our measurements). Type `nt=128↵` to set the number of scans to 128.
- B. TYPE `su↵`. This sets up the experiment, by adjusting the hardware for observing the particular nucleus you have chosen. To display the chosen parameters below the spectrum window, type `dg↵`.
- C. TO BEGIN ACQUISITION, type `ga↵`, or use ACQUIRE menu and click on the appropriate box (e.g.GO, with FT).
- D. TO MONITOR THE STATUS of the experiment you can look at the acquisition status window. To check your spectrum after 16 scans, type `wft↵` after 16 scans have been obtained. A spectrum should appear.
- E. AFTER ACQUISITION IS COMPLETE, use the FULL box to see the full spectrum and PHASE it by typing `aph↵`.

4. Transform, phase, integrate, save and print the ^{13}C spectrum, and then obtain a peak print-out of the ppm values of the resonances.

- A. You should now have the raw data, which will need to be *transformed*. If you would like to view the raw data, type `df↵`. The decaying sine wave (free induction decay, or FID) will show up, which is the so-called "time domain" for your data. To view the data in the "frequency domain", type `wft↵` for "weight fourier transform" and you will see something that looks more like a

- spectrum, although it may be out of phase. This will also dump you into a subroutine, which I call the "display interactive mode". If at any time you are unable to manipulate spectra according to the following directions, click on MAIN MENU, followed by the DISPLAY and then the INTERACTIVE buttons. (cr (cursor), vs (vertical scale) and delta (distance between cursors) at the bottom of the spectrum refer to the three mouse buttons.)
- B. Click on the FULL box to see the full spectrum and *phase* it by typing **aph**↵.
 - C. Use the right and left mouse buttons to put the cursors around the triplet of peaks corresponding to CDCl₃. Click on the button EXPAND on the button bar. This should enable you to clearly see all three peaks. Place the cursor, using the left mouse button on the center peak and set the position of the *reference* by typing **r1(77.00p)**↵.
 - D. *Display* the spectrum as you would like to plot it. Go back to the full spectrum by clicking on the FULL button and then place the cursors on either side of the region of the spectrum that you would like to plot. You can display the scale by typing **dscale**↵ or use the NEXT box to display the DSCALE box and click. You can switch from ppm to Hertz by typing **axis='h'**↵ or **axis='p'**↵. Check the intensity of the spectrum to make sure it is not off scale or too small. Use the middle mouse button and click and drag to adjust. This is a touchy adjustment, so be patient. You may also type **vs=150**↵ and the computer will set the tallest peak of your spectrum to 150 mm.
 - E. Integrate the spectrum or a portion of the spectrum by typing **region**↵ with the appropriate portion of the spectrum displayed. Then click on the PART-INTEGRAL box in the DISPLAY INTERACTIVE menu, to see the integrals.
 - F. To list the peaks on the spectrum or in a table you will want to set the *peak threshold*. Click on the NEXT, then TH buttons and set the yellow line using the left mouse button and clicking or clicking and dragging. Any peak above the line will be recognized as a peak on your spectrum or in your line list. Any peak below this line is considered noise.
 - G. To give your spectrum a *title*, type **atext('whatever you want')**↵.
 - H. Now save the spectrum in your own subdirectory, by first moving to that subdirectory using the MAIN MENU, FILE, SET DIRECTORY buttons as described earlier. Then type **svf**↵ and the computer will prompt you for a name to use to save your file. Or, you may click the FILE, DATA, and then SAVE.FID buttons. To re-display your spectrum, type **ds**↵.
 - I. To send your spectrum to the *plotter*, you may type the commands **pl pscale pap ppf**↵ or use MAIN MENU, DISPLAY, and PLOT buttons; then hit PLOT (again), SCALE, ALL PARAMS and perhaps, PEAKS. The **ppf** command (or PEAKS button) is optional if you don't want to plot the peak frequencies.
 - J. To *print* what's been sent to the plotter, click the PAGE box, or type **page**↵. Nothing will come out of the plotter without entering the page command. Everything you send to the plotter in the interim will be put onto the same sheet of paper. Don't forget this command.

5. Log out properly.

- A. After you are through collecting data, remove your sample and insert the standard sample into the probe. LOCK & SHIM ON THE STANDARD SAMPLE!! DISCONNECT from the acqui window.
- B. To EXIT VNMR, click on the MAIN MENU box, then click on the MORE box. Finally click on the EXIT VNMR box. This will quit VNMR.
- C. To LOGOUT, put the cursor on the wallpaper, hold down the right mouse button, and move the mouse to select **exit**. The computer will ask you to confirm the exit. Click the CONFIRM button.

Lab Report

Your report should include the items listed below.

- I. Each student should make assignments of the spectra to the structures in the handout exercise on which you worked in lab. Analysis of each spectrum, both ^{13}C and DEPT, should be done as well. In cases where signals lie close together, if it was possible to assign them with reasonable certainty, be sure to explain how they were differentiated. If you are unable to assign such signals, this should also be stated clearly. Your rationales can be written on the spectra themselves.
- II. Each student should make assignments of the ^1H NMR signals for carvone and cuminaldehyde. In cases where signals are overlapping, if it was possible to assign them with reasonable certainty, be sure to explain how they were differentiated. If you are unable to assign such signals, this should also be stated clearly. Your rationales can be written on the spectra themselves.
- III. Students should provide one another with data (^{13}C NMR) from the two oils that were isolated by steam distillation. Each student should then independently analyze the data, and assign, to the extent possible, the ^{13}C signals of the oil, based on ^{13}C NMR analyses of carvone, cuminaldehyde, and limonene done in parts (I) and (II). The relative concentration of carvone and cuminaldehyde to limonene in each oil sample should be roughly calculated, by comparing the integration values of *similar types of carbons* from cuminaldehyde or carvone with those of the limonene component.
- IV. Analysis of the IR spectra of cuminaldehyde and carvone should be carried out. Assignments of peaks, which can be made directly on the spectra, should include C=O stretching, C-H stretching of various types (alkyl, vinyl, aromatic, aldehyde), and C=C stretches.
The IR spectra of cumin seed oil and of caraway seed oil should be compared, respectively, with the IR spectra of pure cuminaldehyde and pure carvone, and any major differences noted.
- V. Use the mass of oil that you isolated from either cumin or caraway seeds to calculate the percent by mass of steam-distillable oil in your original sample of seeds.