A Senior-level Experiment in Structural Elucidation Using 2-D NMR

Grant N. Holder,* **David G. Farrar, and David M. Gooden**

Department of Chemistry, Appalachian State University, Boone, NC 28608, holdergn@appstate.edu

Abstract: One-dimensional (^{1}H, ^{13}C, DEPT), and two-dimensional (COSY, HETCOR) NMR experiments are used to assemble the chemical structures of monoterpene unknowns assigned to senior instrumental analysis students. Students were asked to use their knowledge of NMR to deduce the structure of their assigned compound.

Introduction

One of the more useful tools for the organic or analytical chemist is NMR spectroscopy; however, in many cases, as was true in our own program, exposure is limited to simple ^{1}H acquisition of elementary systems, generally substituted aromatics or organics. Having recently acquired a Varian 300-MHz Gemini instrument through the National Science Foundation's ILI program, we were anxious to provide a more thorough example of NMR spectroscopy than time-tested (and still useful) experiments such as keto–enol tautomerism. We were concerned that our students (in senior-level instrumental analysis) be exposed to some of the more common 2-D techniques.

Fuson [1] has proposed an NMR module for an instrumental analysis course, but his focus is on the mechanics of obtaining spectra: signal digitization, quadrature detection, data processing etc. These topics are covered in our senior-level NMR spectroscopy course. Modern NMR software, along with standardized pulse sequences and optimized parameters, has rendered the acquisition of quality spectra a routine matter, provided that a "resident spectroscopist" periodically calibrates the instrument and updates the appropriate files. We, therefore, have chosen to focus on the applications side of NMR spectroscopy, giving attention to structure elucidation of natural products [2].

Mills [3] proposed an experiment utilizing the 2-D COSY experiment along with molecular modeling to assign proton resonances in several bicyclic monoterpenes. In these experiments, however, the structures of the terpenes are known to the students, and only one experiment beyond the basic ^{1}H experiment is explored. Roark and Mosher [4] extended Mills' experiment by introducing delay COSY to look at long-range coupling. Again, however, the structures of the bicyclic molecules are known and only one additional experiment is introduced.

We propose an experiment in which students must deduce the structure of an unknown monoterpene using commonly available NMR experiments.

Experimental

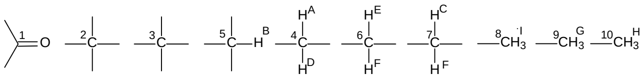
Each student is assigned an unknown and given two prepared samples (in $CDCl_3$), a "dilute" sample and a "concentrated" sample. The dilute sample is used to obtain the ^{1}H-detected spectra (^{1}H, COSY-45) while the concentrated sample is used to obtain the ^{13}C-detected spectra (^{13}C, DEPT, HETCOR). This approach enables the student to obtain all the spectra in a single day. Students then schedule times to acquire the ^{1}H-detected spectra and the ^{13}C-detected spectra.

The students are given instruction on each of the experiments during the lecture portion of the course. In addition to interpretation, they are also given an overview of each experiment. This includes the difference between COSY-90 and COSY-45 in terms of pulse sequence, parameters and resulting spectra, and the importance of an accurately calibrated 90° pulse in the decoupler channel for DEPT and HETCOR experiments.

With the spectra in hand, the student is faced with the real challenge, interpreting their data. The students are encouraged to start with the ^{1}H and ^{13}C spectra to obtain a basic C_mH_n formula (realizing that hetero atoms may be present) and to make general classifications of the protons and carbons as aromatic, vinylic, aliphatic, etc. For example, with camphor the ^{1}H-spectrum (Figure 1) indicates that all proton resonances are aliphatic. The three upfield singlets (labeled G, H, and I in Figure 1) suggest the presence of three methyl ($-CH_3$) groups. Integration gives 16 protons. The ^{13}C spectrum (Figure 2) shows 10 ^{13}C resonances, 9 aliphatic resonances, and 1 downfield resonance at approximately 220ppm that suggests a ketone carbon (see Figure 2). At this point the student has the following pieces to the structure puzzle: (1) the chemical formula is $C_{10}H_{16}O$, (2) there is a probable $>C=O$, and (3) three methyl groups are present.

The next step is to examine the DEPT spectrum (Figure 3). This allows the student to categorize the carbon resonances as quaternary, methine, methylene, and/or methyl. Analysis of the camphor DEPT spectrum confirms the previous assessment of 3 $-CH_3$ groups. It also adds two quaternary carbons to the fragment list in addition to the carbonyl, methine, and three methylene carbons. The proton count for these fragments is 16, indicating that all the proton resonances are due to C–H bonds. This confirms the previously determined formula, $C_{10}H_{16}O$, and points to a structure with three degrees of unsaturation. The absence of vinylic resonances in both the proton and carbon spectra indicates that two rings are present in addition to the known double bond ($>C=O$).

With the various puzzle pieces in hand, the next step is to determine the connectivity of those pieces. The tools for this task are HETCOR and COSY experiments. HETCOR (Figure 4) provides the student with ^{1}H-^{13}C correlation; for example, it shows that proton B is directly attached to carbon 5. The HETCOR experiment, in essence, assigns labels to the fragments; thus, the HETCOR experiment updates the list of puzzle pieces to:



The COSY experiment (Figure 5) provides information about proton–proton correlations, which protons are within, typically, two to three bonds of one another, although long-range (four-bond) coupling is occasionally evident. Indeed, four-bond coupling between the

© 1999 Springer-Verlag New York, Inc., S1430-4171(99)05329-2, 10.1007/s00897990329a, 450173gh.pdf

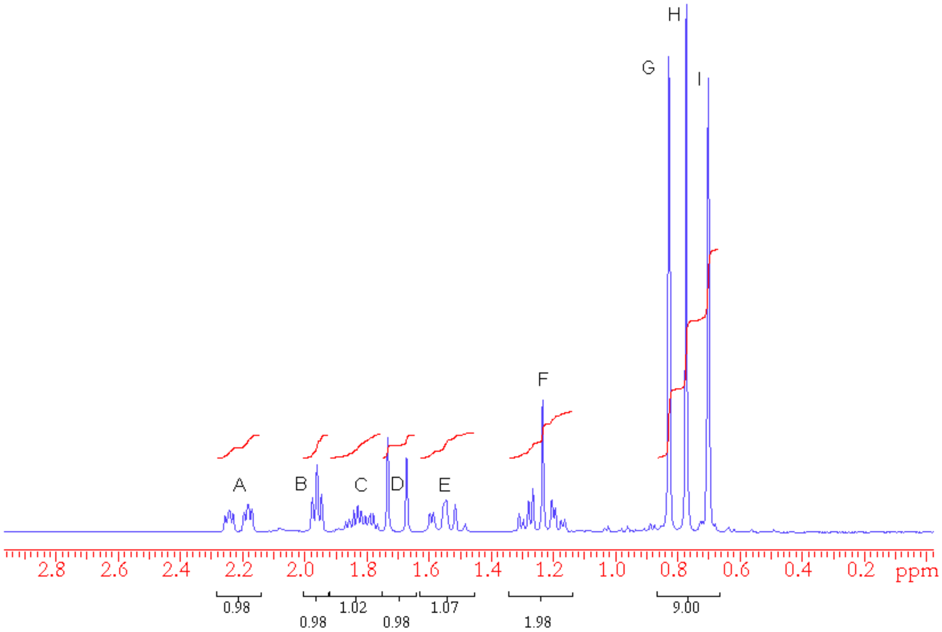


Figure 1. ^{1}H-NMR spectrum of camphor.

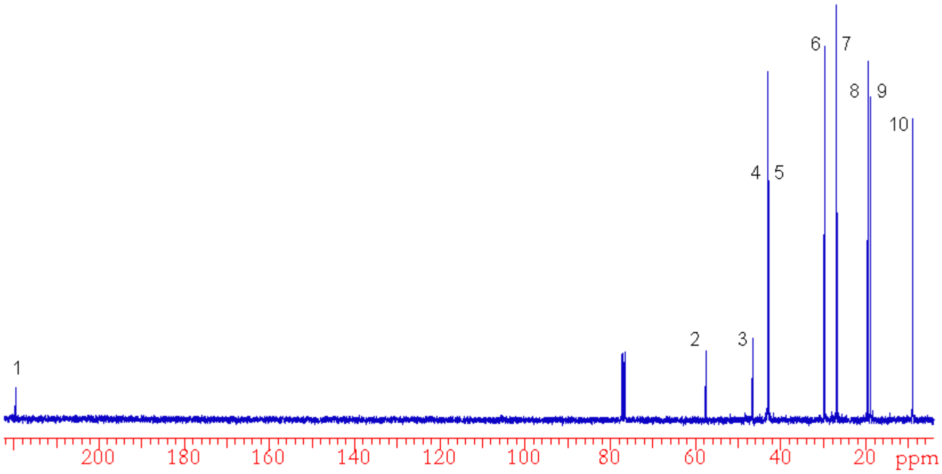


Figure 2. ^{13}C-NMR spectrum of camphor.

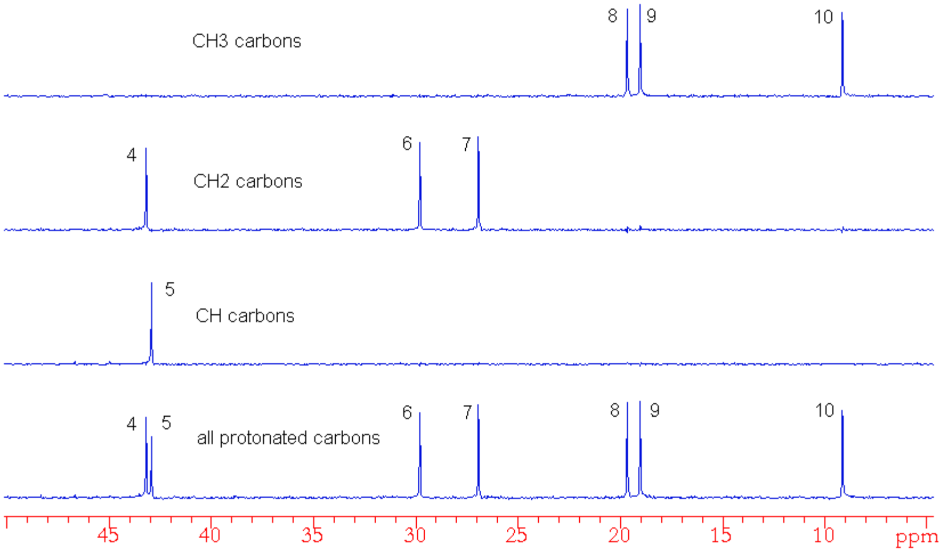


Figure 3. DEPT spectrum of camphor. Peak labels correspond to those of the ^{13}C spectrum.

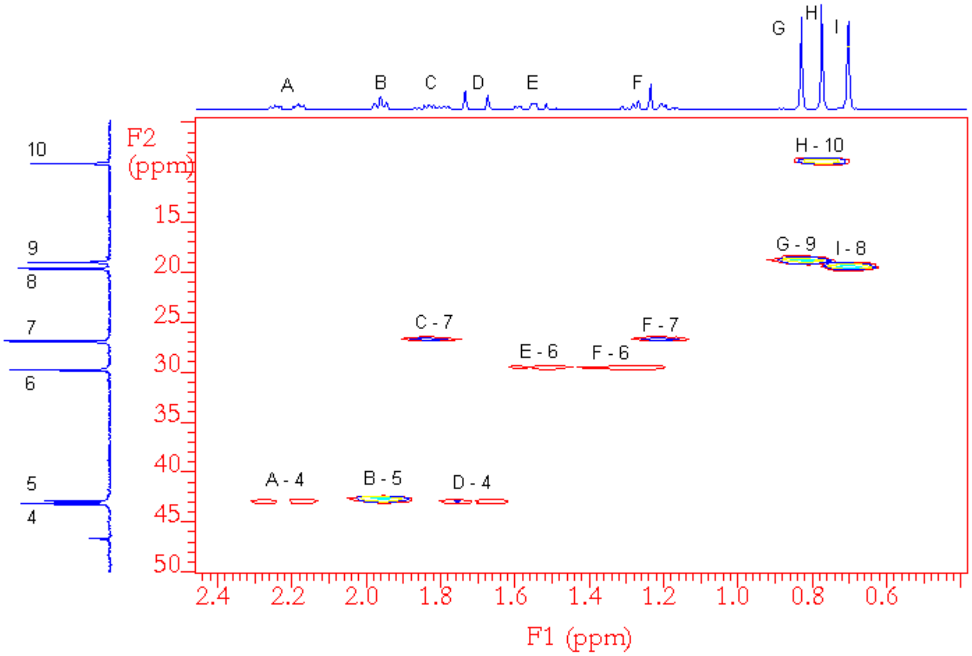


Figure 4. HETCOR spectrum of camphor. 1-D spectra along F1 and F2 are the high-resolution spectra shown in Figures 1 and 2. Peak labels correspond to those of the high-resolution spectra.

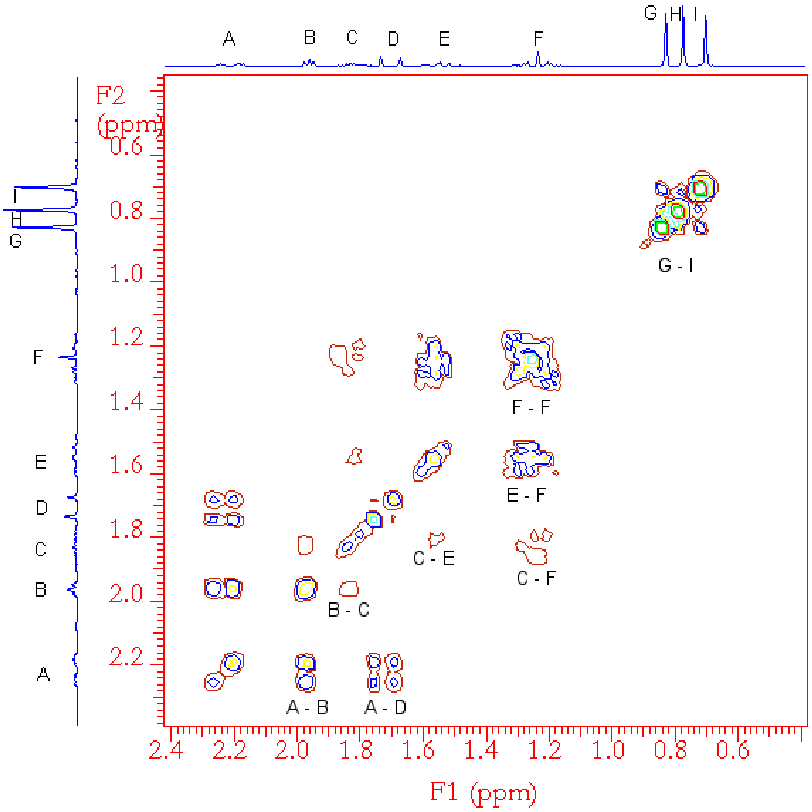
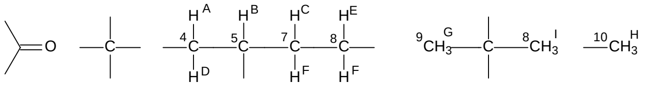
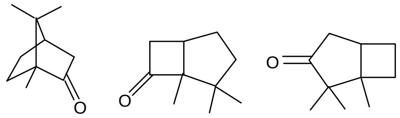


Figure 5. COSY spectrum of camphor. 1-D spectrum is the high-resolution spectrum shown in Figure 1. Peak labels correspond to those of the ^{1}H spectrum.

hydrogens of two of the methyl groups is present in the COSY spectrum of camphor. The students must rationalize that this coupling cannot be three-bond coupling because that would indicate that the methyl groups are directly bonded, giving ethane. The students can further deduce that, because the methyl signals are singlets, the connecting carbon must be one of the quaternary carbons. The student now has the following assemblage:



Based on chemical shift, the most upfield proton (and the corresponding carbon) is adjacent to the carbonyl moiety. Bearing in mind that the compound is bicyclic, the possibilities for assembling the remaining fragments is greatly limited. The authors could find only three.



It is not unreasonable to present the students with a problem that does not have a single, unique answer. Not only will the students encounter such problems in their careers, but also it is instructional for them to realize that other experiments may be necessary to unambiguously assign a structure.

Discussion

The decision to use monoterpenes was predicated on several factors: cost, safety, complexity, and solubility. Many monoterpenes are commercially available at relatively low cost. While sample sizes are small, providing a diverse sample array requires purchasing a number of different terpenes. With 10 carbon atoms, monoterpenes are large enough to present the students with an interesting challenge without being unduly complex. The monoterpenes generally display excellent solubility (often miscible) with common organic NMR solvents such as $CDCl_3$. This permits the preparation of highly concentrated samples, allowing the acquisition of all the carbon-detected spectra within a two-to-four-hour time span.

With some compounds, the aliphatic envelope involves significant overlap, making interpretation excessively difficult. One option is to use another solvent. For example, the ^1H spectrum of α-terpineol in $CDCl_3$ has excessive overlap in the aliphatic region, rendering interpretation of the COSY and HETCOR spectra unwieldy. Recording the spectrum in acetone-d_6, however, resolves the peaks sufficiently that interpretation of the COSY and HETCOR spectra are relatively straightforward.

One complication that the authors encountered is the presence of long-range (four-bond) coupling in the COSY spectra of some of the bicyclic terpenes. This coupling was especially prevalent between the bridgehead protons of the terpenes containing the 1,1,3 bicyclic framework. Given their experience and the methods available to them, the students generally will be unable to distinguish four-bond coupling from three-bond coupling. Instructors must be aware of pitfalls such as this and either indicate such peaks in the COSY spectra or refrain from assigning unknowns of this type.

Four-bond coupling consequent to long-range interactions across a double bond does not present a problem. The students are instructed that such coupling is not uncommon. Because vinylic protons and carbons are readily identifiable, this aids rather than hinders the process of structural elucidation.

Cineole, which contains an ether oxygen in the bicyclic framework, is an excellent candidate for this experiment. While the ^{13}C chemical shifts suggest the presence of the ethereal oxygen, there is no direct evidence for it. For this reason, one may wish to provide the students with mass spectral data to confirm the presence of the oxygen. The existence of the –OH group in alcohol-containing compounds is indicated, first, by the ^{13}C chemical shift and the inconsistency of the proton count provided by the ^1H spectrum and, second, by the DEPT spectrum. Generally, mass spectral data are unnecessary, but such data do not trivialize the students' task.

While some terpenes, such as menthol, α-terpinene ,and linalool, allow a complete unambiguous structural determination, others are not so well behaved. One option is to accept that students may present more than one possible structure or that some students may require a little additional information. A second option is to introduce other experiments, such as COLOC (to show long-range, 2–3 bond, H–C correlations) or INADEQUATE (to show C–C correlations). Using 70–80% solutions of monoterpene in $CDCl_3$, we have been able to obtain INADEQUATE spectra showing most of the C–C correlations in 12 hours. With a moderate-to-large class size, however, even this could represent a prohibitive amount of spectrometer time. Furthermore, from a pedagogical standpoint, the INADEQUATE experiment is so powerful it trivializes much of the reasoning process required by the COSY experiment, often obviating the need for the COSY. Also, not all of the terpenes show sufficient solubility to obtain an INADEQUATE spectrum in a reasonable time frame.

As with other instrumental techniques (GC–MS, HPLC, IR, etc) the output could be used as a stand-alone interpretation exercise, eliminating the data collection and processing from the exercise. While that may be necessary in some situations, the authors feel that hands on experience with instrumentation is an important aspect of chemistry students' education.

Conclusion

This experiment provides students with broad exposure to the diverse techniques available using NMR spectroscopy. It also challenges students to logically assemble the various "puzzle pieces" provided by each technique to develop a cohesive picture of the structure.

Supplementary Information

Table compiling 18 monoterpenes and an assessment of the suitability of each terpene for this experiment is available in the supporting material (2 pages) (45gh1897.pdf).

References

1. Fuson, M. M. *J. Chem. Educ.* **1994**, *71*, 126.
2. For resources on NMR interpretation and instrument operation see: (a) Sanders, B. K. M.; Hunter, B. K. *Modern NMR Spectroscopy: A Guide for Chemists*; Oxford University Press: Oxford, 1993; (b) Braun, S.; Kalinowski, H.-O.; Berger, S. *150 And More Basic NMR Experiments: A Practical Course*; Wiley: New York, 1998; (c) Martin, G. E.; Zektzer, A. S. *Two-Dimensional NMR Methods for Establishing Molecular Connectivity: A Chemist's Guide to Experiment Selection, Performance, and Interpretation*; VCH Publishers: New York, 1988; (d) Derome A. E. *Modern NMR Techniques for Chemistry Research*; Pergamon Press: New York, 1987.
3. Mills, N. S. *J. Chem. Educ.* **1996**, *73*, 1190.
4. Roark, J. L.; Mosher, M. D. *Chem. Educator* **1998**, *3*(1): S1430-4171(98) 04229-6. Avail. URL: http://journals.springer-ny.com/chedr.