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Laboratories and Demonstrations

An Advanced Undergraduate Experiment in 2-D NMR

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The concurrent use of one-dimensional (^{1}H-NMR) and two-dimensional (^{1}H-^{1}H-COSY and ^{1}H-^{1}H-Delay-COSY) experiments can unambiguously assign each resonance in a spectrum to a particular proton. We have developed an experiment for the advanced chemistry student that utilizes long-range coupling to assign signals in the spectra of ketopinic acid, borneol, and isoborneol. Prelaboratory discussion of the spectra of camphor, hands-on use of the instrumentation, and determination of molecular stereochemistry are topics visited during the course of this experiment.

The students received hands-on use in obtaining two-dimensional spectra….

Introduction

Vicinal and geminal ^{1}H-NMR coupling constants have been a major tool for the establishment of proximity relationships and relative stereochemistries in natural and synthetic organic

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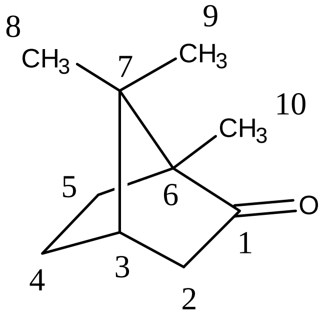

FIGURE 1. *dl*-CAMPHOR.

molecules. More distant molecular relationships have been verified by a number of techniques including nuclear Overhauser effects [1, 2], T_1 relaxation times [3], and shift reagents [4]. The use of long range proton coupling, particularly ^4J "W coupling," to establish structural features separated by several bonds has been used in many cases [5]. We wish to report an experiment we use in our instrumental analysis course that illustrates the ease of establishing the presence of small couplings over four or five bonds with 2-D ^{1}H-^{1}H correlation spectroscopy (COSY) [6] and the power of this measurement in structure assignment [7].

Mills [8] recently reported a novel, advanced NMR experiment that illustrates the power of COSY in the complete assignment of the ^{1}H signals in verbenone, myrtenal, myrtenol, and α-pinene. The COSY spectra of these compounds readily allow for the assignment of all signals in these molecules with the exception of the bridgehead methyls. To quote [8]: "COSY analysis is unable to resolve this ambiguity." The assignments of the bridgehead methyls were originally made with the aid of lanthanide shift reagents [9]; Mills [8] reports the conformation of these assignments with NOESY experiments.

We have used a similar experiment in our organic spectroscopy course in recent years using a similarly rigid molecule, *dl*-camphor (Figure 1); however, we have been able to unambiguously assign all ^{1}H signals, including the bridgehead methyl hydrogens, using COSY spectroscopy. Furthermore, we have confirmed that this method can be extended to similar bicyclo[2.2.1]heptanes.

Figure 2 shows a contour plot of the two-dimensional data set obtained from the COSY spectrum of *dl*-camphor in deuterochloroform. The corresponding one-

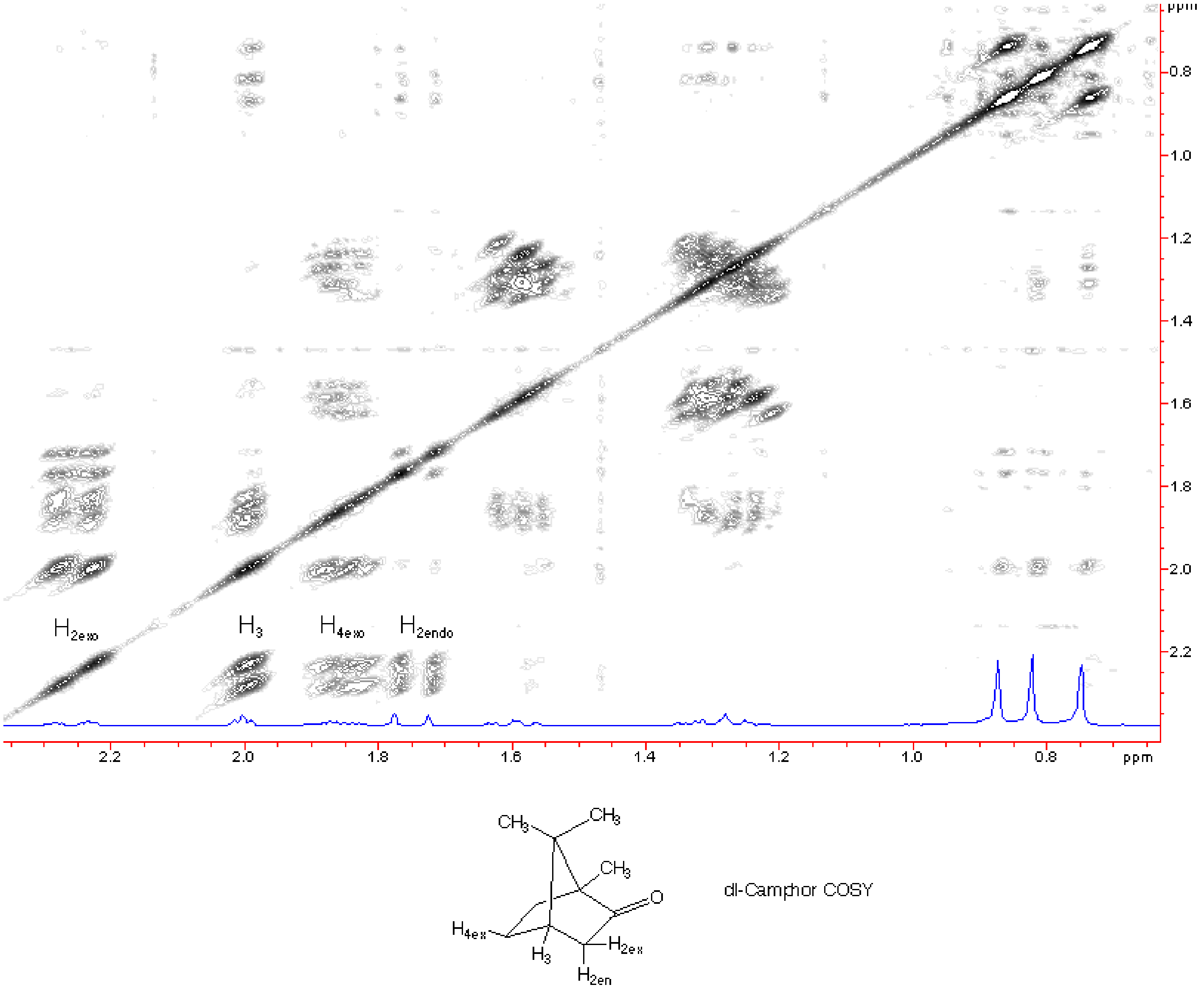

FIGURE 2. *dl*-CAMPHOR COSY CONTOUR PLOT.

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dimensional spectrum is shown along the ordinate. The downfield signal in the spectrum ($\delta = 2.2$) must be $H2_{exo}$ or $H2_{endo}$ given the proximity of these two hydrogens to the carbonyl. That it is $H2_{exo}$ can be established by the pattern of this signal in the one-dimensional spectrum which clearly shows the presence of coupling to three hydrogens, to H3 and $H2_{endo}$ as expected, and to $H4_{exo}$ *via* ^4J W coupling. In this COSY spectrum the low-field signal has three cross peaks, which correspond to coupling to H3 ($\delta = 2.0$), $H2_{endo}$ ($\delta = 1.8$), and $H4_{exo}$ ($\delta = 1.9$).

These assignments are confirmed by the one-dimensional signals for $H2_{endo}$ and H3. The former signal in the one-dimensional NMR is a doublet eve though coupling might be expected to the geminal $H2_{exo}$ and the vicinal H3. Because the vicinal dihedral angle is approximately 90º, this coupling is approximately 0 Hz [10].

Similarly, the dihedral angle between H3 and $H4_{endo}$ is approximately 90º, thus this coupling constant is also 0 Hz. Consequently, H3 is only coupled to two of four neighbors, and the signal is observed to be a triplet (double doublet). The assignments for the remaining secondary hydrogen signals readily follow from these initial assignments although it is to be noted that the signals for $H4_{endo}$ and $H5_{endo}$ essentially overlap. (This overlap can be removed by recording the COSY spectrum in perdeuteroacetone.)

The assignments of the methyl signals can be made from the Delay-COSY spectrum in Figure 3 [11]. The upfield and downfield methyl signals are connected by cross peaks and are the geminal pair. The coupling constant for this system has been estimated [12] to be in the range 0.1–0.5 Hz and is observable in the one-dimensional spectrum as line broadening. Cross peaks are also observed for the ^5J coupling between H3 and all three methyl signals, $H4_{endo}$ and $H5_{endo}$ (the spectrum in perdeuteroacetone confirms this statement) and the high-field methyl, and between $H2_{endo}$ and the downfield methyl. All of these couplings are considerably less than 1.0 Hz and are not at all apparent in the one-dimensional spectrum of *dl*-camphor. Similar ^5J couplings have been noted in other systems and strongly suggest the structural feature shown in Figure 4 [7].

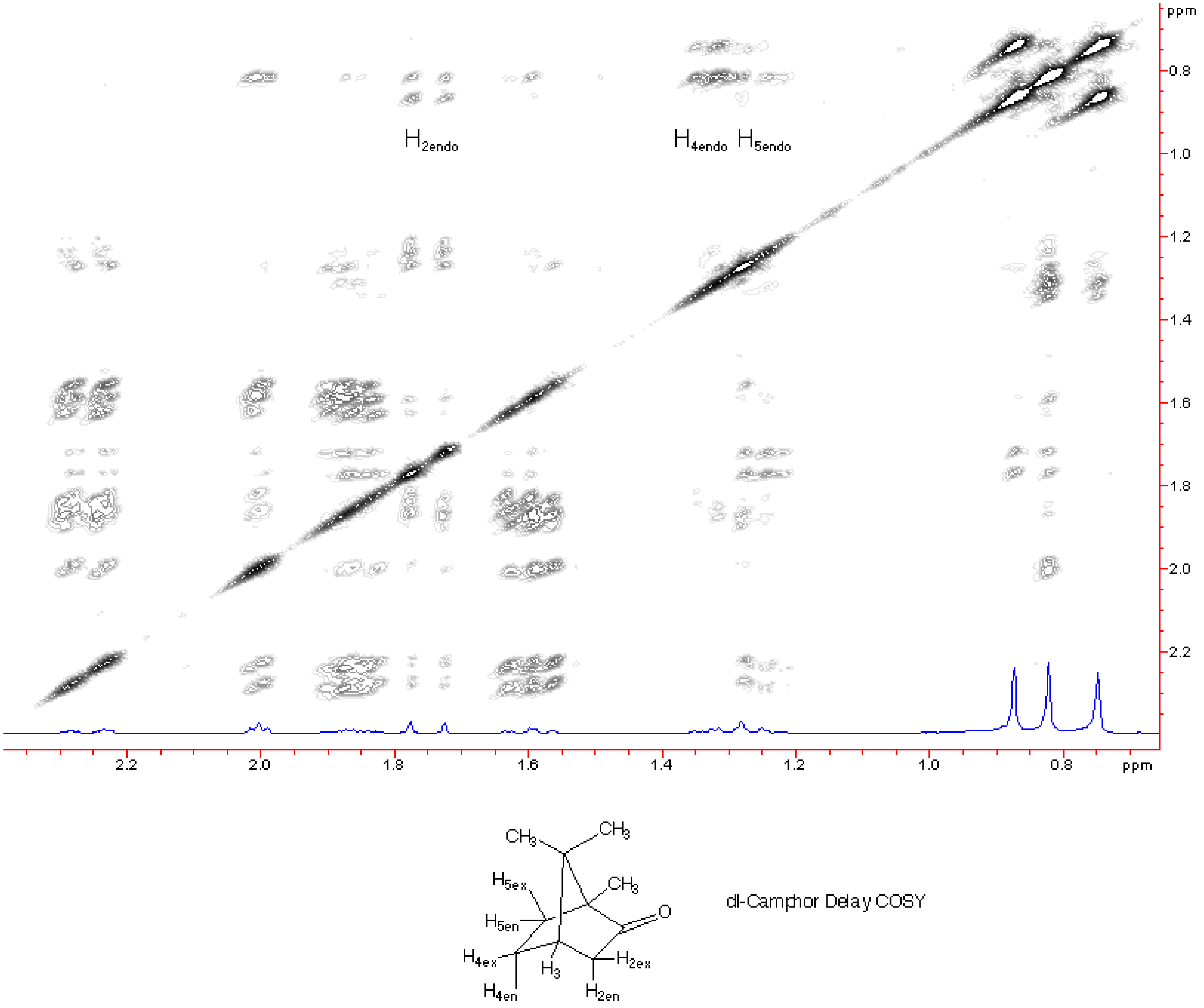

FIGURE 3. *dl*-CAMPHOR DELAY-COSY CONTOUR PLOT.

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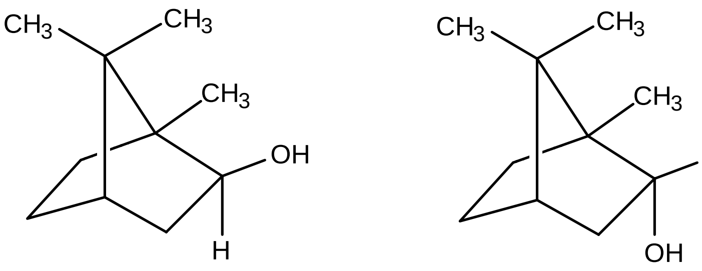
FIGURE 4. ^5J PSEUDO-W.

The assignments of the geminal methyls follow. The methyl coupled to H2$_{endo}$ is the opposite bridgehead methyl, C8 methyl, and the C9 methyl is the one coupled to H-4$_{endo}$ and H5$_{endo}$. These identical assignments were made previously by comparisons with model systems and careful measurements of line broadening [5]. The direct observation of these long range couplings allows for the definitive proton assignments of *dl*-camphor in two COSY experiments. Even though no direct information is available from these experiments as to the magnitude of the coupling constant, a purely pragmatic interpretation of the data is sufficient for structure assignment.

While the camphor spectra serve to illustrate many important NMR concepts for the advanced student, we have chosen a related set of compounds, *d*-ketopinic acid, *l*-borneol, and *dl*-isoborneol, to illustrate these concepts through laboratory experimentation. Use of racemic mixtures instead of the individual isomers was guided by the cost of purchasing the particular compound. No differences are observed in the NMR between racemic mixtures and enantiomerically pure compounds.

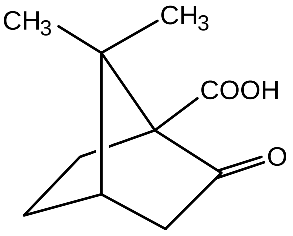
Experimental

Free-induction-decay information for each compound was obtained in deuterochloroform on a Bruker WM-360 (360 MHz for ^{1}H, widebore magnet) unless otherwise indicated. COSY and Delay-COSY experiments used a 90° pulse followed by a 45° pulse to reduce the height of signals along the diagonal. The Delay-COSY experiment is also known as a Long-Range-COSY experiment [1]. The pulse sequence is $\pi/2 - \Delta - t_1 - \pi/4 - \Delta - t_2$, where $\pi/2$ is the 90° pulse, $\pi/4$ is the 45° pulse, and Δ is a fixed delay introduced into the mixing times t_1 and t_2. For the experiments described in this paper, Δ was chosen as 0.20 s. Data were transferred to a Power Macintosh 7200/120, transformed into spectra using MacNUTS, and printed for evaluation. Chemical shifts were reported relative to $(CH_3)_4Si$ and residual nondeuterated solvent. *dl*-Camphor, *d*-ketopinic acid, *l*-borneol, and *dl*-isoborneol were purchased from Aldrich Chemical Company and used without purification.



Isoborneol

Borneol



Ketopinic Acid

FIGURE 5. *d*-KETOPINIC ACID, *l*-BORNEOL, AND *dl*-ISOBORNEOL.

Results and Discussion

d-Ketopinic acid was chosen as the starting point for the advanced student due to the fact that its ^{1}H-NMR and COSY spectra are much simpler than for *dl*-camphor. Each signal is fairly well resolved at 360 MHz when the sample is dissolved in deuterochloroform. The students obtained the spectra for *d*-ketopinic acid and assigned each of the signals based on the spectral interpretation of *dl*-camphor given in the prelaboratory lecture.

Structural features of *d*-ketopinic acid similar to *dl*-camphor are readily apparent (see Figure 5); $H2_{exo}$ is a doublet of doublets of doublets via vicinal, geminal, and ^4J W coupling. $H2_{endo}$ appears to be coupled only to $H2_{exo}$. The COSY spectrum of *d*-ketopinic acid can be used to unambiguously assign the bridgehead methyl protons (Figure 6). Because of the geometry constraints for ^5J long-range coupling, one of the

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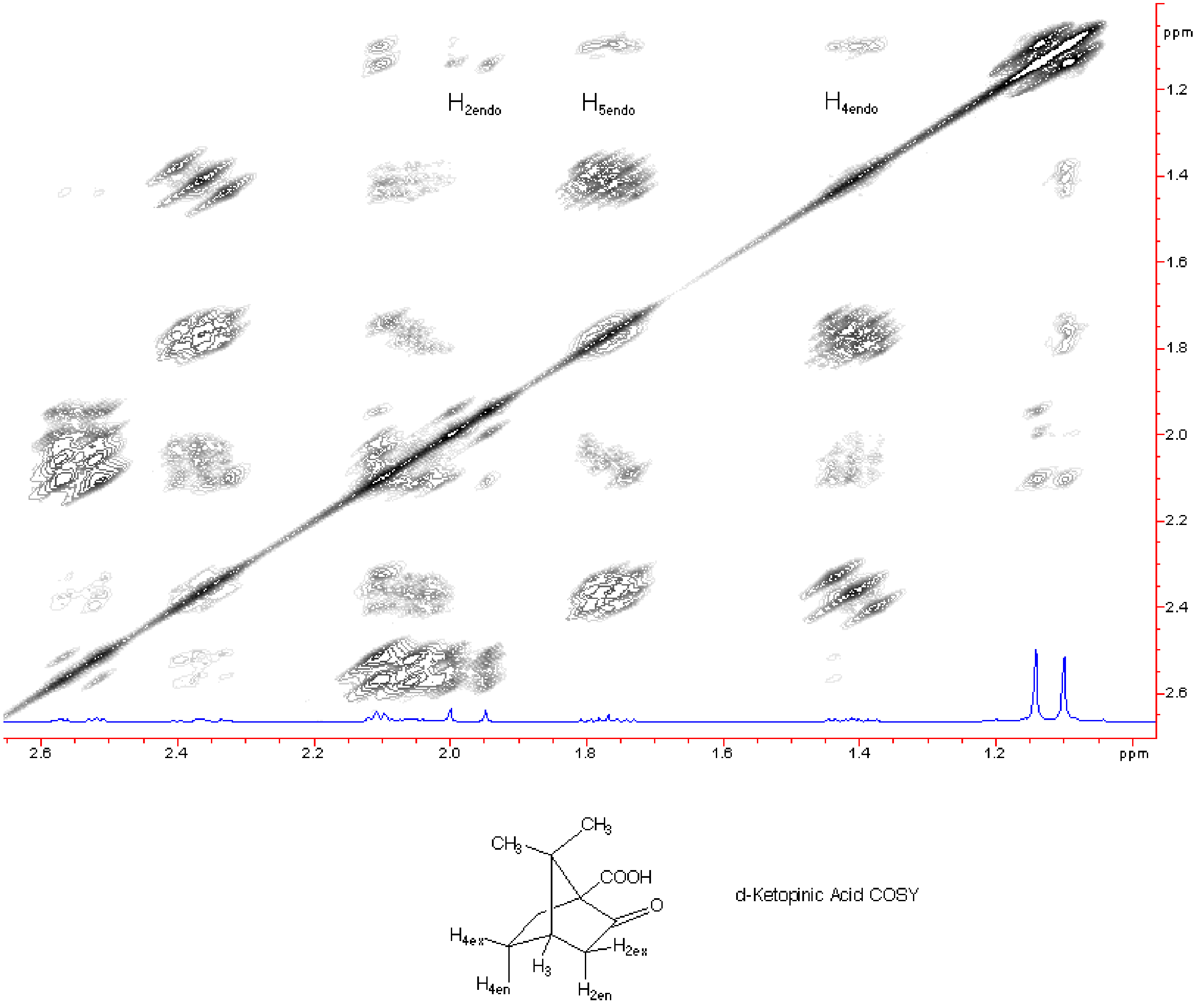

FIGURE 6. *d*-KETOPINIC ACID COSY CONTOUR PLOT.

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methyl groups is coupled only to $H2_{endo}$ and H3 while the other is coupled only to $H4_{endo}$, $H5_{endo}$, and H3.

This information is used by the students to unambiguously assign the stereochemistry in the borneol system. The students were then given two unknowns in unlabeled NMR tubes: *l*-borneol and *dl*-isoborneol, each dissolved in deuterochloroform, and told to identify them. The students then obtained a ^{1}H-NMR, a COSY, and a Delay-COSY spectrum for each of the unknowns at 360 MHz.

Despite the fact that the three methyl groups are not well resolved, the Delay COSY of *l*-borneol easily provides the answer to the puzzle (Figure 7). The most downfield signal, $H1_{exo}$ (δ = 3.94), is coupled to $H5_{exo}$ via a long-range W coupling. This indicates that *l*-borneol must possess an endo hydroxyl group. This fact is confirmed by examination of the *dl*-isoborneol COSY spectrum (Figure 8). Despite the lack of resolution of most of the signals, $H1_{endo}$ (δ = 3.59), is coupled via a ^5J pseudo-W to one of the methyl groups, and via a ^4J W to the bridgehead methyl.

Conclusion

The students were able to assign each of the protons in *d*-ketopinic acid to a signal in the ^{1}H-NMR through use of the COSY and Delay-COSY experiments. Application of this knowledge, based on the presentation of the assignments of *dl*-camphor, to the problem of identifying an unknown substituted bicyclo[2.2.1]heptane was readily accomplished via these two-dimensional NMR experiments. The students received hands-on use in obtaining two-dimensional spectra, learned the basics of signal generation and reception in the NMR, and were reacquainted with many one-dimensional experiments (determination of T_1, one-dimensional ^{1}H NMR, determination of 90° pulse width, etc.).

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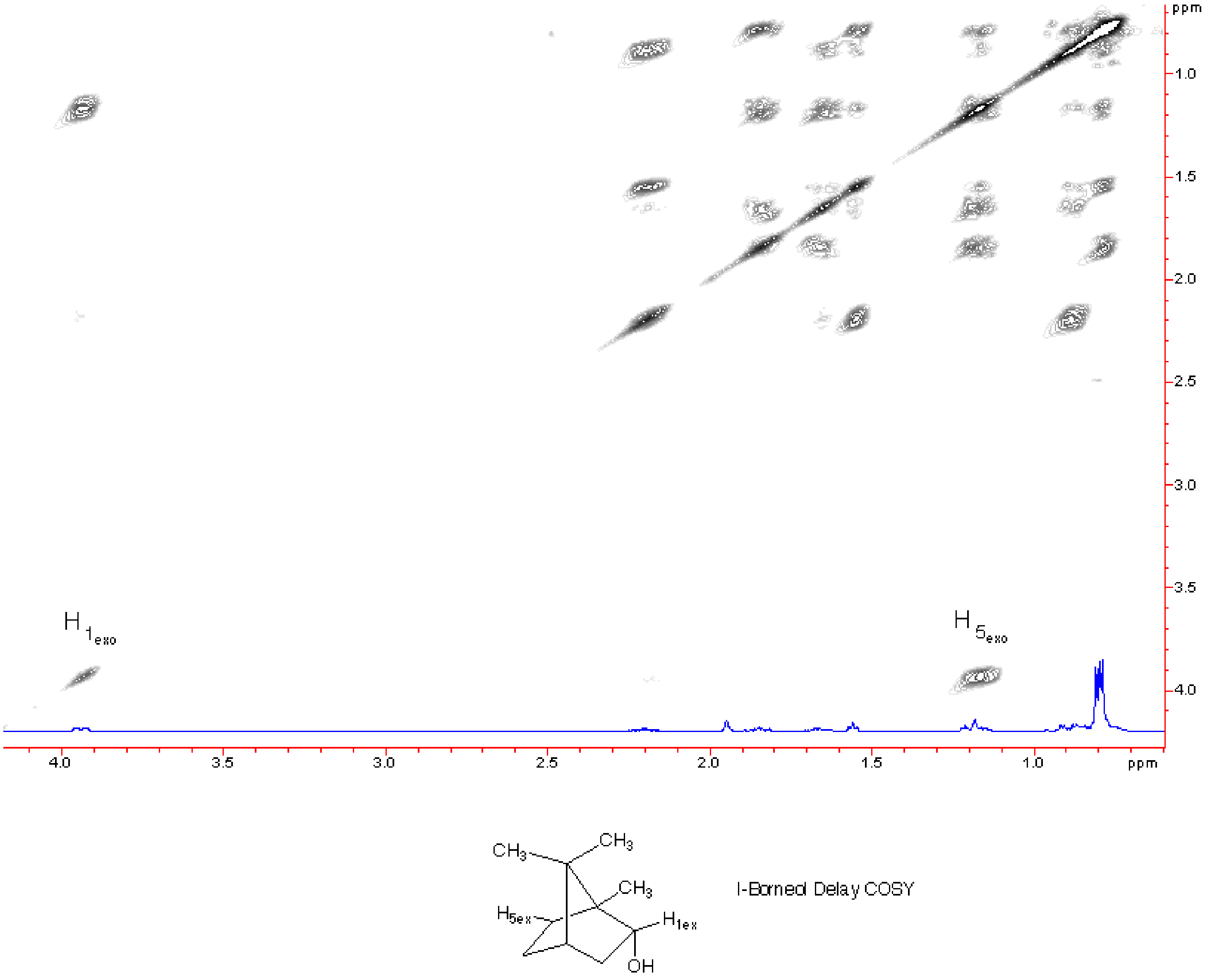

FIGURE 7. *l*-BORNEOL DELAY COSY CONTOUR PLOT

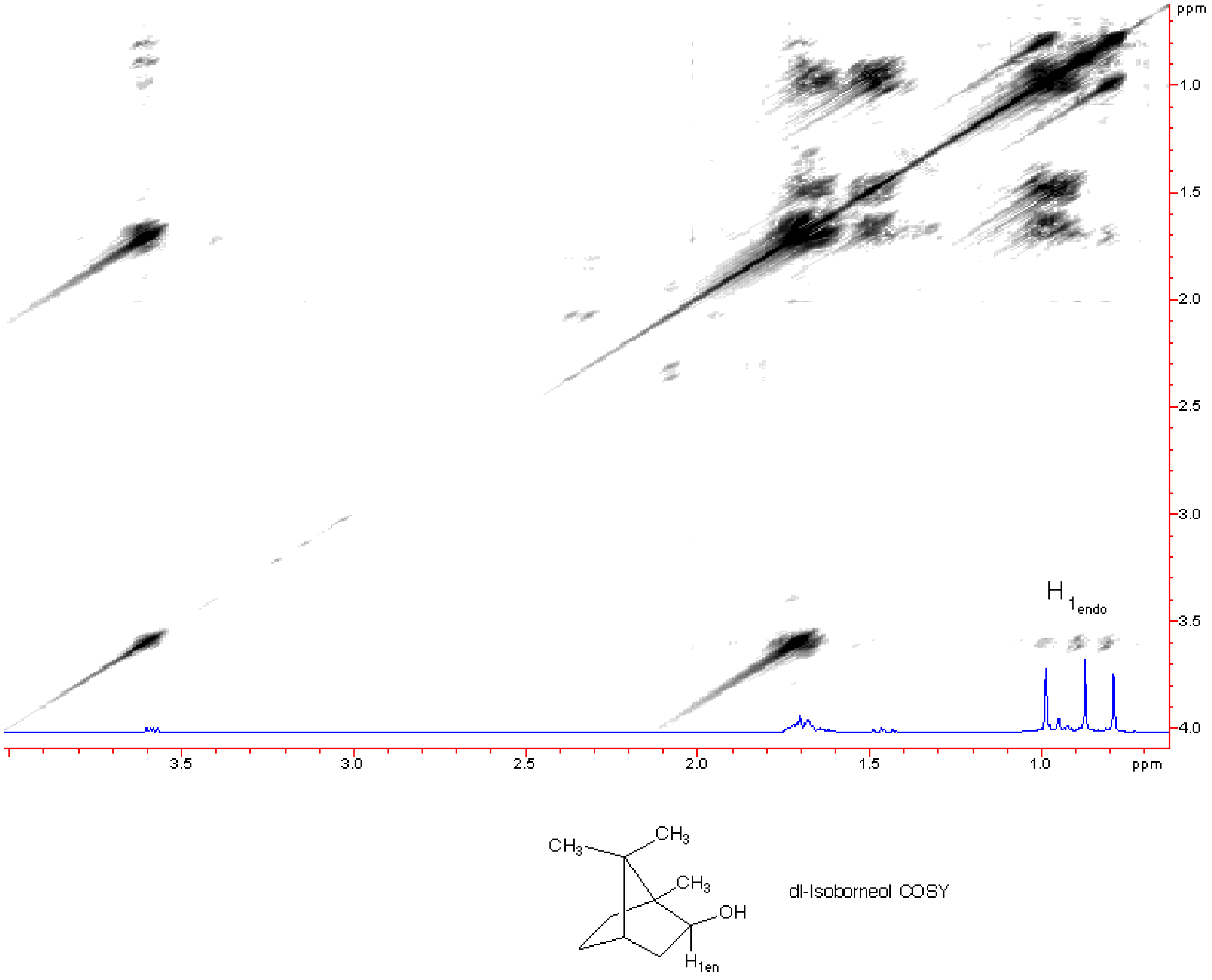

FIGURE 8. *dl*-ISOBORNEOL COSY CONTOUR PLOT.

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