NMR Deconvolution: Quantitative Profiling of Isomeric Mixtures

Lauren S. Lopansri, Jacob N. Letson, Richard A. O'Brien, David R. Battiste, David C. Forbes*

Department of Chemistry, University of South Alabama, Mobile, AL 36688, USA
*Corresponding author: dforbes@southalabama.edu

Abstract A method involving NMR band deconvolution has been applied to the instructional/research activities at the undergraduate level. Three case studies are presented to illustrate how unresolved peaks of ^{1}H and ^{13}C NMR spectra can used to obtain quantitative data on isomeric composition. The data generation is rapid and the method can be applied to any organic chemistry laboratory curriculum/research program involving the analysis of NMR spectra. The incorporation of NMR band deconvolutions offers an ideal platform to transition from qualitative to quantitative discernment of NMR data.

Keywords: *second-term organic chemistry, isomeric analysis, NMR band deconvolution*

Cite This Article: Lauren S. Lopansri, Jacob N. Letson, Richard A. O'Brien, David R. Battiste, and David C. Forbes, "NMR Deconvolution: Quantitative Profiling of Isomeric Mixtures." *World Journal of Chemical Education*, vol. 10, no. 2 (2022): 51-61. doi: 10.12691/wjce-10-2-1.

1. Introduction

Ask any seasoned organic chemistry laboratory instructor or member of a synthetic organic lab involving undergraduate researchers: Obtaining just not qualitative but quantitative data offers the ideal setting when wanting to hone one's ability to interpret NMR spectra [1-6]. This is especially true as the vast majority of those acquiring the spectra are doing so have just been formally introduced to the concept. Pedagogically, when focusing on just the lab of organic chemistry, it is through spectroscopy that one has the best opportunity to provide evidence-based experiments which reinforces the goals of that experience [7-10]. A classic example is the formation of 2-chloro-2-methylbutane from the corresponding tertiary alcohol using concentrated hydrochloric acid (Scheme 1) [11].
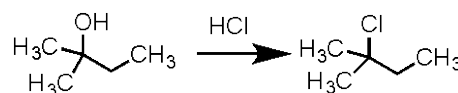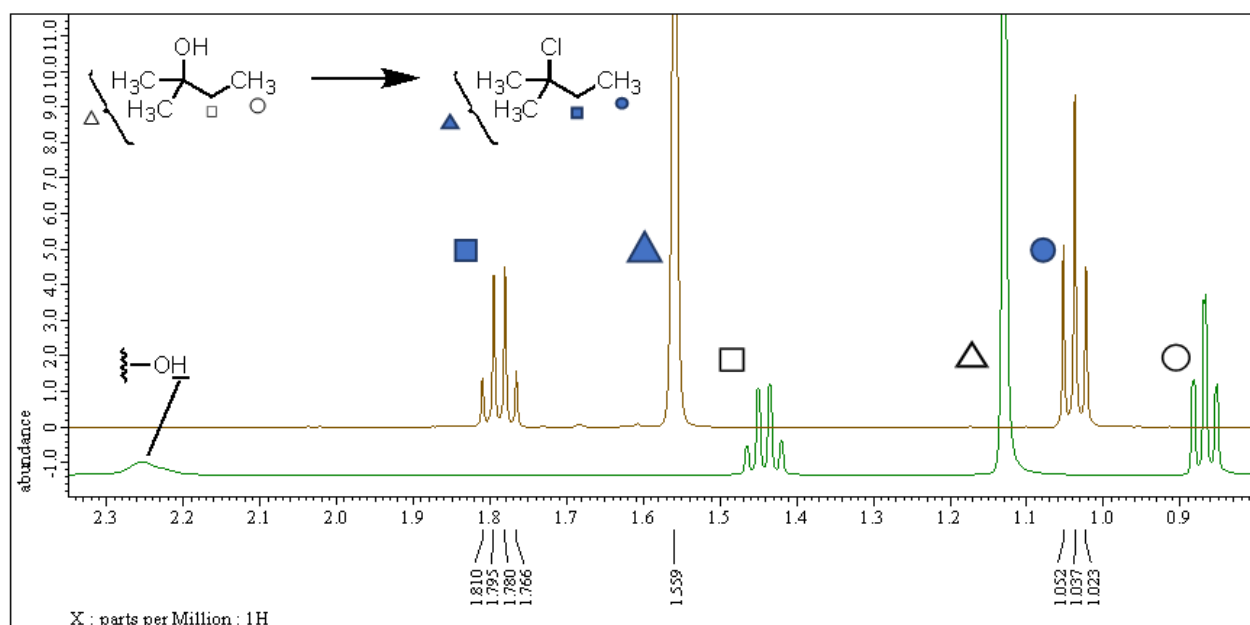
Scheme 1.

Figure 1. ^{1}H NMR spectra of 2-chloro-2-methyl butane (filled) and 2-hydroxy-2-methylbutane (unfilled)

For this experience, one has the opportunity to confirm product formation by boiling point, IR spectroscopy, and NMR spectroscopy. Unique with this ^{1}H NMR analysis is the fact that all the peaks, not just the diagnostic peaks, of both starting material and product are resolved (Figure 1). Outside of the fact that one must tease out the 6H integrals representing the geminal methyl groups (filled and unfilled triangles in Figure 1), this example offers everyone the opportunity to both qualitatively and quantitatively comment on product formation and thus reinforce what arguably is one of the most challenging topics in organic chemistry, spectroscopy.

What is interesting is that for the very next experiment of our curriculum [12], students are asked to perform an E1 reaction having just submitted their post laboratory reports commenting on and providing evidence of product formation of 2-chloro-2-methylbutane via a S_N1 process. For the latter, the experiment involves an acid catalyzed dehydration of 4-methylcyclohexanol [11] and the same rubric of submitting an evidence-based post laboratory report is asked of those enrolled. While it is clear that the absence of hydroxy functionality in the IR spectrum offers key evidence of product formation, what is observed, unfortunately, with both the discussions and conclusions of the post laboratory reports submitted is far more qualitative rather than a quantitative NMR analysis (Figure 2).
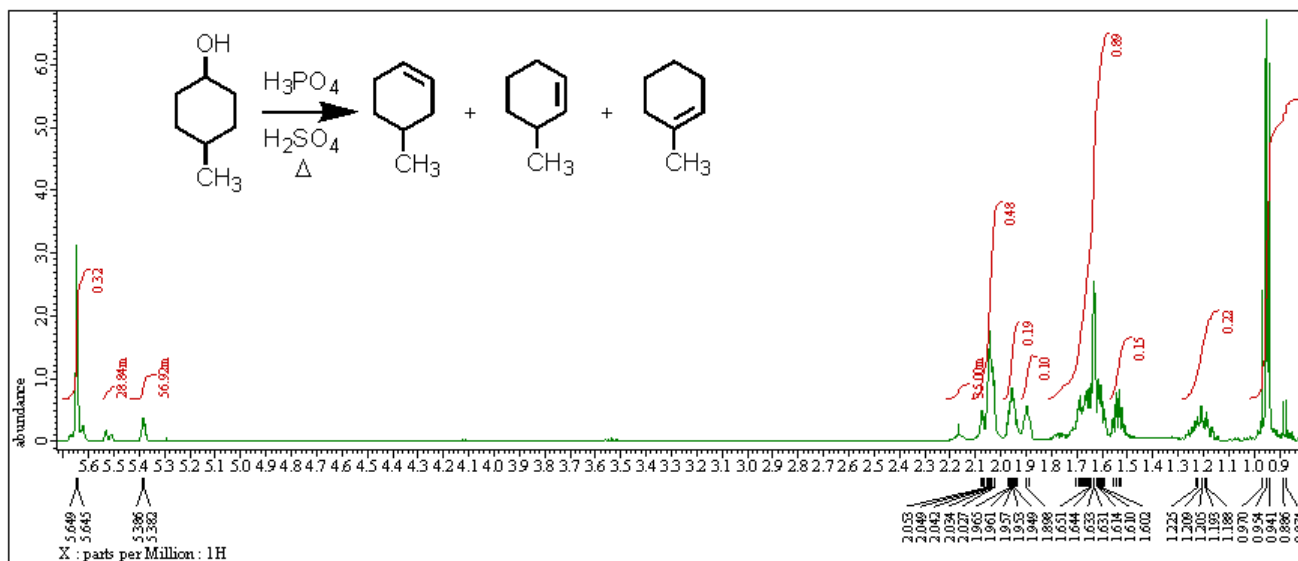

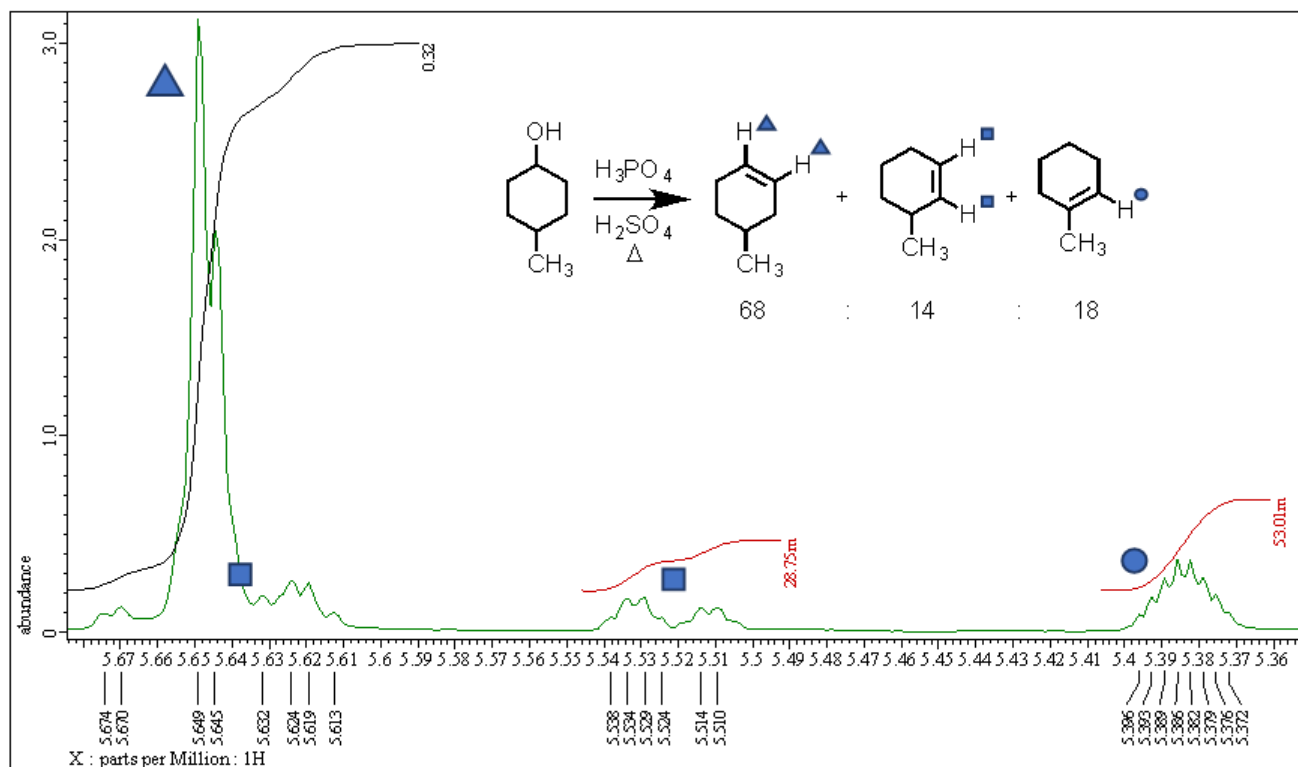**Figure 2**. Qualitative evidence of alkene formation using ^{1}H NMR

Figure 3. Quantitative evidence of alkene composition using ^{1}H NMR spectroscopy

In the absence of a GC chromatogram, a quantitative analysis would typically begin by expanding the vinylic region of ^{1}H NMR spectrum where evidence of all three isomers of methylcyclohexene exists (Figure 3). That is, upon obtaining the diagnostic chemical shifts of each isomer from the literature, the isomeric composition can be both confirmed and quantified within this region. Analyses of this type truly allow for the experimentalist to develop their critical thinking skills while reinforcing a number of key concepts presented in the lecture setting which for this example includes intermediate/product stability as isomeric mixtures are formed. Thus the challenge, especially for those teaching a second-year instructional lab, is placing an emphasis on spectral interpretation having as the goal one obtaining and then commenting on quantitative data obtained using the resources available.

2. Background

Recognizing the importance of quantitative analyses, members of the Department of Chemistry at the University of South Alabama have for over five years integrated and continually improved upon our approach toward evidence-based experimentation in the organic laboratory. The broader impact is significant as this format of data analysis is one of the core deliverables of our first- and second-term organic chemistry laboratory experiences which serves just over 500 students per year representing all the disciplines of the institution. Students upon isolating a product, determine the percent yield and prepare their NMR samples either prior to or soon after performing an IR analysis. Once placed into a carousel which allows us to act upon an automated sequence and run of all the samples for an entire section, electronic and hard copies of ^{1}H and ^{13}C spectra are generated for the entire section usually within 45 minutes. In many instances, students leaving a particular lab session have an IR spectrum confirming functional group manipulation and ^{1}H and ^{13}C spectra offering additional support of product formation.

The use of NMR spectroscopy, especially qNMR, in the organic curriculum is far from new and the number of articles/communications having this tool successfully integrated into the instructional lab is both extensive and growing [13-16]. The common thread with each is the opportunity to delve deeper into NMR analyses. This should not be surprising given recent advancements in both NMR hardware and software capabilities [17,18]. Opportunities now can be applied to any organic chemistry laboratory curriculum and perhaps more importantly any synthetic organic research program involving undergraduate researchers having as the goal, again, to delve deeper into their NMR analyses. The reality of NMR spectroscopy, however, is that not every spectrum obtained will fall under the category of crisp, resolved, and easily interpretable as is the case illustrated in Figure 1. The step function involved in our own organic curriculum is steep and clearly evident when comparing Figure 1 and Figure 2. This is especially true when conducting experiments with undergraduate researchers

involving method development having as the goal improving elements of reaction outcome, such as, conversion or isomeric composition.

Outside of the fact that deciphering NMR spectra is an acquired skill and many of those enrolled or perhaps starting a research experience are just learning the concept of spectroscopy, a rapid method which allows one to keep NMR as the primary tool for analysis would be most beneficial. As such, an opportunity exists to assist in the deciphering of NMR spectra using software advancements as many encounter situations where the material they spent hours making consists of unresolved peaks. The best case which illustrates this point is when the experimentalist is confronted with isomeric mixtures where the key analysis must be of the crude reaction mixture. Presented herein are three case studies illustrating the viability of band deconvolution fitting when confronted with spectra consisting of unresolved peaks of crude isomeric mixtures.

3. Method

For each example, JEOL Delta[TM] Version 5.0.4.4 (PC) or 5.2.1 (MAC) was used to run the deconvolution of peaks [19]. The Delta software is free, allows the user to process the data generated on any PC (Windows and Mac), and if necessary, the opportunity to get/register/operate 1D data processing exists via web seminars hosted by JEOL. Furthermore, the standard operating procedures for performing band deconvolutions are both known and readily available for Agilent/Varian, Bruker, and JEOL. Both ^{1}H and ^{13}C NMR spectra were used for the rapid discernment of isomeric ratios of crude reaction mixtures. For the isomeric mixtures which were not separated, the data to confirm each isomer from the crude spectra was obtained using literature chemical shifts of the respective isomer(s). Once the peaks were identified, the integrals obtained were used to calculate the percent composition of each isomer, that is, relative isomeric compositions were determined. Not determined and outside the scope of this study was an absolute quantitative analysis, qNMR using an internal reference and error analysis.

4. Hazards and Safety

Our standard operating procedure (SOP) applies to all instructional and research laboratory activities. The SOP outlines specifics concerning conduct, proper handling of chemicals, attire, eye protection, and proper handling of equipment [20]. All the chemicals and materials used for the synthetic procedures were stored, dispensed, and properly disposed of under a laboratory fume hood or an approved designated area within the instructional/research space. For this article, an inclusive list of the flammable/hazardous materials used are as follows: 1,3-Butadiene, *trans*-2-butenoic acid (crotonic acid), camphor, deuterated chloroform, ethyl acetate, diethyl ether, hexanes, hydrochloric acid, 2-hydroxy-2-methylbutane, 4-methylcyclohexanol, methylene chloride, 2-methylpropene (isobutylene), phosphoric acid, sodium borohydride, sulfuric acid, zinc chloride, and zinc sulfate.

5. Results and Discussion

5.1. Case Study: Crude Reaction Mixtures

For the purpose of this case study, the hydrochlorination of a cyclohexene derivative consisting of two pre-existing stereocenters was examined. The goal was to improve both regio- and diastereoselection. This project was part of a Senior Honors Thesis [21] and addresses a core concept presented in every organic chemistry lecture experience, that being, additions across sites of unsaturation. Specific to this project was the role of zinc promoters in hydrochlorination reactions of *trans*-6-methylcyclohex-3-enecarboxylic acid which when esterified as the *tert*-butyl ester produces the attractant Trimedlure (Scheme 2).

Trimedlure has been shown to be one of the most successful attractants discovered for the Mediterranean fruit fly, or medfly, to identify infestations and alert growers to spray for these invasive pests. Over 100,000 traps and lures containing Trimedlure are used annually in the United States in an effort to combat medfly infestation to fruit and vegetable crops. What is very interesting about the material used is both the stereochemical makeup and composition. Approximately 95% of the isomeric composition of Trimedlure consists of a racemic mixture of four diastereo- and regioisomeric isomers of chloromethylcyclohexanecarboxylic acid as the *tert*-butyl ester. One isomer, (1*R*, 2*R*, 4*S*)-*tert*-butyl 4-chloro-2-methylcyclohexanecarboxylate, consists of 38-45% of the mixture and has been found to be the most active attractant of the eight used. The key synthetic step as shown in Scheme 2 is the hydrochlorination of *trans*-6-methylcyclohex-3-enecarboxylic acid. Represented in Chart 1 are the four regio- and diastereomeric isomers observed in this step. The most active attractant as the *tert*-butyl ester stems from carboxylic acid **3**.

Because both GC/MS and NMR analysis of the hydrochlorinated isomers yielded data with unresolved peaks, we opted to go with the latter as the data generated could be compared to the chemical shifts obtained from the literature of all four racemates independently prepared, isolated, and characterized. A representative spectrum using NMR band deconvolution methodology is below (Figure 4).
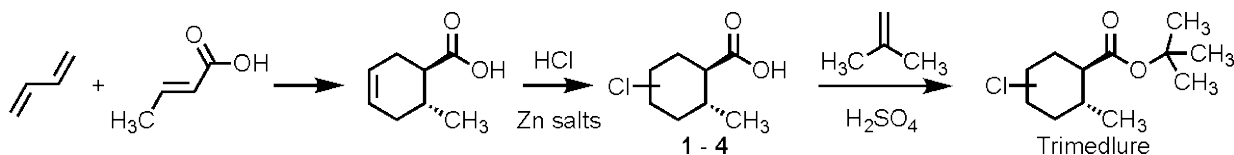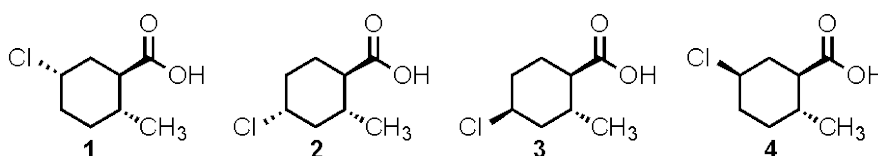
Scheme 2.

Chart 1. Possible regio- and diastereoisomers generated in the hydrochlorination of *trans*-6-methylcyclohex-3-enecarboxylic acid
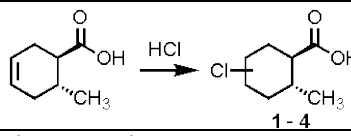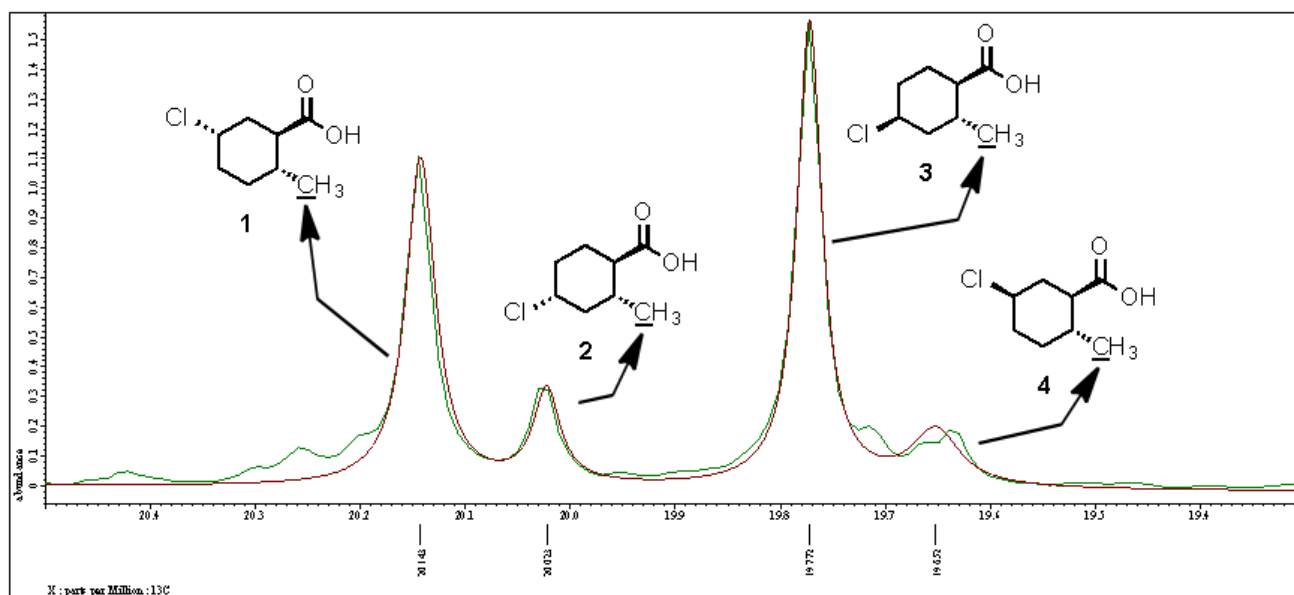
Figure 4. ^{13}C NMR band deconvolution of isomeric peaks in absence of zinc promoter

The expanded ^{13}C NMR spectrum of Figure 4 focuses on just the 19 - 20 ppm range and clearly illustrates a hydrochlorinated product revealing four identifiable peaks of the methyl group off of C-2 of cyclohexyl ring. Once marked, the peaks were deconvoluted. For this example, NMR band deconvolution was necessary because the trendline was only possible when looking at the mixture of isomers of the crude reaction mixture. As such, the minor isomer which often encountered low signal to noise and/or spurious peaks was cyclohexyl derivative **4**.

Table 1 provides the data for one of the hydrochlorination reactions of *trans*-6-methylcyclohex-3-enecarboxylic acid without zinc promoter. In addition to listing the peak assignments as they correspond to the isomers presented in Figure 4, the isomeric composition was determined using the integrals obtained.
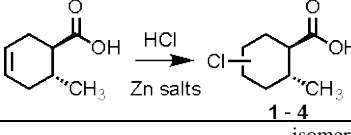
Table 1. Isomeric composition using band deconvolution method

hydrochlorinated adduct	peak (ppm)	integral	isomeric composition
1	20.143	5.535	36
2	20.023	1.477	10
3	19.772	6.699	44
4	19.652	1.545	10

Advantages with a case study where NMR spectra are obtained on crude reaction mixtures are two-fold. First, even with spectra consisting of low signal to noise resulting in peaks which are truncated or the presence of spurious peaks as is the case here, the depth of literature data available to match known diagnostic peaks is arguably the highest when compared to spectra generated from other standard instrumentation such as GC, GC/MS, IR, LC, and LC/MS. Second, with situations where low signal to noise/truncation was observed (e.g. 19.652 ppm of Figure 4), the data generated establishes a *baseline from which trendlines can be documented*. Table 2 presents the data in this study using zinc promoters. The isomeric compositions represent values which are averages of no less than three hydrochlorination reactions. In each example, complete conversion of the alkene to the alkyl chloride was observed. When taken collectively, the data generated was rapid, easy, and clearly offers the opportunity to establish quantitative trendlines. While one may view some of the isomeric data here within experimental error, the methodology without question offers a rapid assessment tool using crude material.

Table 2. Isomeric composition in the presence and absence of zinc promoters[a]

entry	promoter	isomeric composition			
		1	2	3	4
1	ZnSO$_4$ • 7H$_2$O	29	9	41	20
2	anhydrous ZnSO$_4$	33	13	43	9
3	anhydrous ZnCl$_2$	35	12	48	6
4	none	36	12	46	7

[a]Data represents averages of no less than three runs.

Notable as well, when considering broader impact, is how this transformation which is presented in the lecture setting of a second-year undergraduate experience does not even apply the Markovnikov/anti-Markovnikov principle per se yet is a real world example involving the use of isomeric mixtures having as the goal the eradication of the medfly which if not monitored, has the capability of costing United States agricultural interest millions of dollars every year. Once prepared, the final step prior to baiting the medfly traps is the esterification of the isomeric carboxylic acid using isobutylene to form the *tert*-butyl ester. As the threat of medfly infestation is becoming more prevalent due to increased importing of agricultural products, this project was conducted in conjunction with the United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) continued action plan for the eradication of the medfly through the use of insect attractants.

5.2. Case Study: Unresolved Peaks

The application of NMR band deconvolution is not limited to trendlines using crude reaction mixtures as shown above. What is arguably the most common use of deconvolution methodology when processing data is its application when encountering peaks which are not resolved.

The second example illustrating an acid catalyzed dehydration of a secondary alcohol[11] which generates not one but three geometric isomers has within the ^{1}H NMR spectrum two distinct regions where one could apply this methodology. The peaks of interest are within the vinylic region and the upfield methyl resonances (Figure 2).

This example highlights however several limitations of NMR band deconvolutions. First, when focusing on Figure 3, specifically the 5.67 – 5.61 ppm region of the ^{1}H NMR spectrum, the vinylic protons of both 4-methylcyclohexene and 3-methylcyclohexene are represented in this region. However, what is unfortunate is that the further downfield multiplet representing one of the vinylic protons of 3-methylcyclohexene is buried underneath the broad peak representing the two vinylic protons of 4-methylcyclohexene. Second and perhaps more discouraging is the extent of unresolved peaks of the aliphatic region outside of the methyl resonances (Figure 2). Third, if wanting to use 13C NMR data, use of integrals without proper controls because of the nuclear Overhauser effect and the low abundance of the carbon-13 isotope can result in misleading data.

All three limitations with this methodology are evident with this example: The vinylic multiplets (Figure 3), the coalesced aliphatic region (Figure 2), and finally an isomeric composition of 68:14:18 using the ^{1}H NMR data compared to a composition of 79:13:8 when using ^{13}C NMR data. However, even with limitations such as these, the opportunity of a qualitative analysis of the vinylic carbons for all three isomers does exist and is presented in Figure 5. Once marked (filled and unfilled circles, squares, and triangles), the resolved signals could be compared to the chemical shifts obtained from the literature of all three alkenes independently prepared, isolated, and characterized.
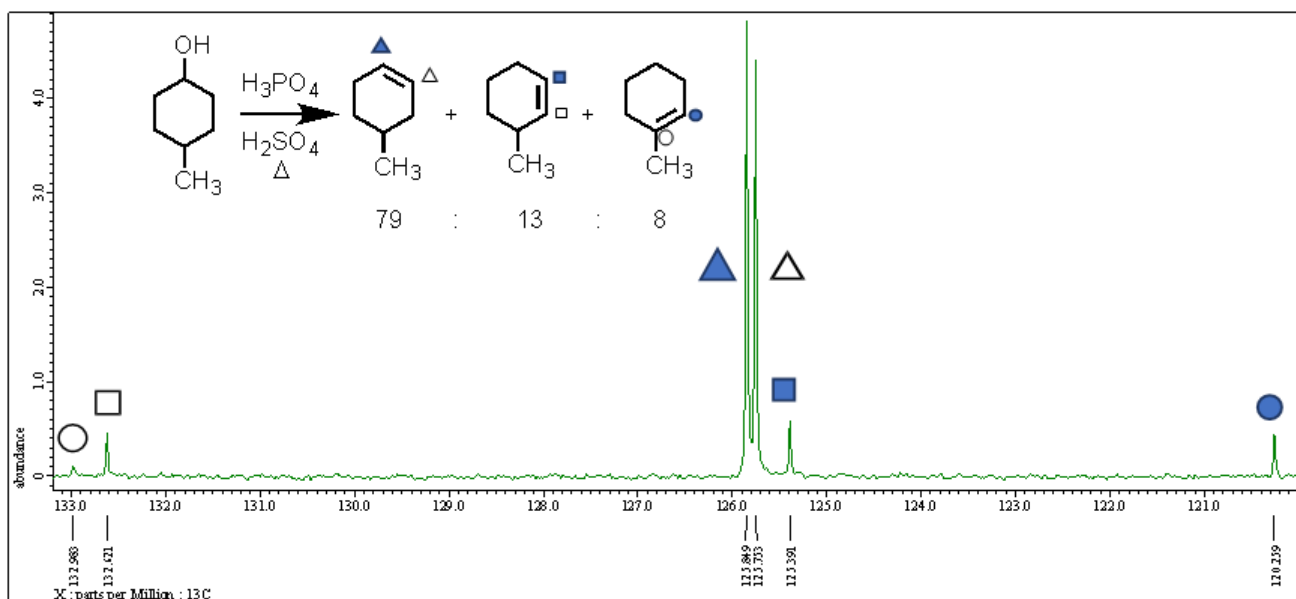
Figure 5. Vinyl region representing all three isomers of methylcyclohexene

Even with limitations, obtaining quantitative data of isomeric mixtures using both ^{1}H and ^{13}C NMR data is possible. The key advantage with ^{13}C NMR as shown in the first case study is that the standard spectra generated are in a decoupled format. That is, information of neighboring protons is not part of the spectrum and the lines displayed are diagnostic for each carbon. Exploiting this fact can be advantageous especially in situations where isomeric mixtures are present thus an ideal platform for deconvolution methodology.

The first step when considering NMR band deconvolution using both the ^{1}H and ^{13}C NMR data is confirmation that the isomeric composition from the ^{1}H NMR coincides with that obtained using ^{13}C NMR. Once confirmed, the opportunity exists to utilize both sets of data and is the focus of the second case study illustrating the opportunity to delve deeper into NMR analyses using the ^{13}C NMR data of unresolved peaks.
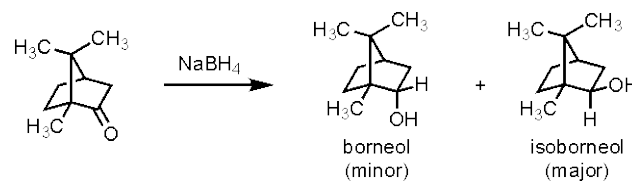
Scheme 3.

Scheme 3 presents the reaction of camphor with sodium borohydride to yield the isomeric mixture of borneol and isoborneol [11]. The isomeric composition of approximately 1:4 was confirmed using the quantitative data obtain of both resolved and unresolved peaks observed in the ^{1}H and ^{13}C NMR spectra.
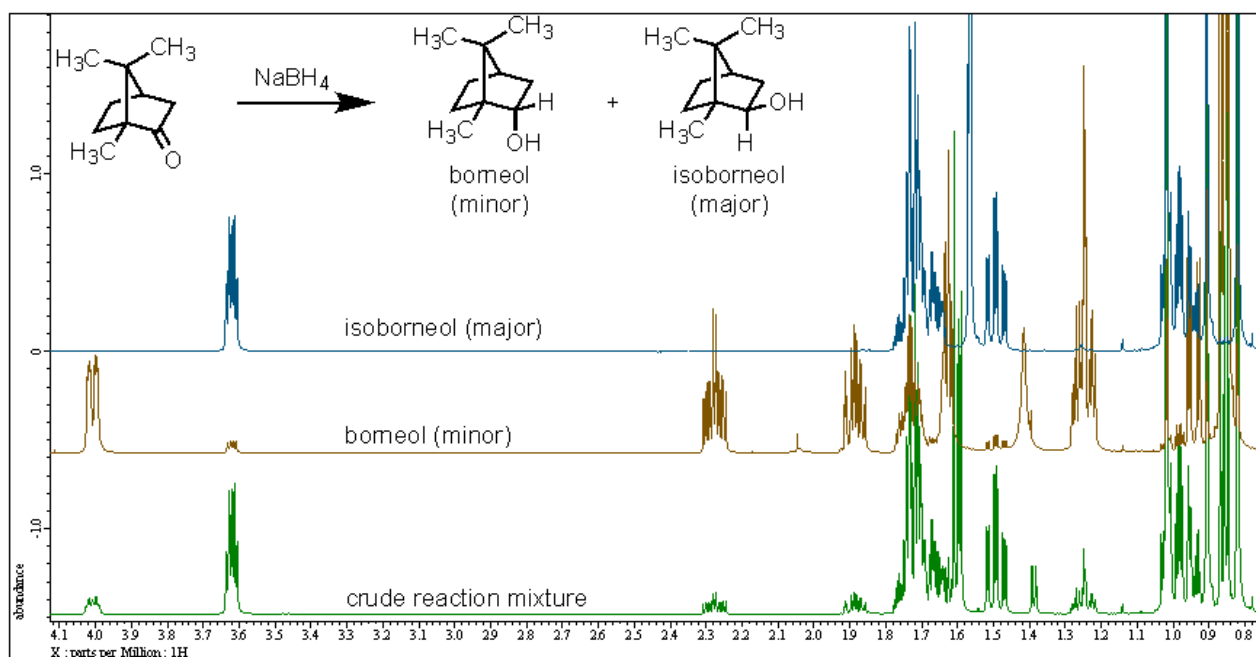

Figure 6. Stack plot of crude and separated/enriched samples of borneol and isoborneol.

What is interesting with this example is the appearance of unresolved peaks in both the purified samples and crude samples of borneol/isoborneol. Figure 6 is a stack plot of the crude reaction mixture illustrating the presence of unresolved peaks in the crude sample, the major isomer isoborneol, and the minor enriched isomer of borneol.

As is the case in the formation of 2-chloro-2-methylbutane and the isomeric mixtures of methylcyclohexane, the reduction of camphor is part of our organic chemistry laboratory sequence (second term) which asks students to analyze the IR, ^{1}H NMR, and ^{13}C NMR spectra they generate. Several terms ago we recognized the need to offer a platform from which expanded regions of the spectra for each sample prepared were part of the assays conducted. Below in Figure 7 is a representative expanded ^{1}H NMR region each student obtains when conducting this experiment. As a result of the return seen in the post lab reports having expanded regions as part of the standard operating procedure for the organic laboratory curriculum, we have increased the number of expanded regions when NMR spectra are generated using the automated sequence to five. As the number of those enrolled in the organic chemistry lab is

just over 500 per academic year, we are excited about reaching our goal of us asking students to offer additional evidence-based commentary by delving deeper into their analyses of the data generated in their post laboratory reports.

While successful with generating quantitative data using the diagnostic region of 3.50 - 4.10 ppm of the ^{1}H NMR spectra in the camphor reduction experiment, there is still a missed opportunity when considering not a qualitative but a quantitative analysis of the NMR spectra generated for this reaction.

Figure 8 below presents the ^{13}C NMR spectrum of the crude borneol/isoborneol mixture and three expanded regions directly below the full spectrum which clearly illustrate opportunities of NMR band deconvolution. For the furthest downfield unresolved expanded region, it is interesting in how the methine carbon of borneol at 77.394 ppm is part of the solvent peak when using CDCl$_3$. The next two expanded sets illustrate unresolved peaks of the minor and major chemical shifts of borneol and isoborneol, respectively. Regardless of what is resolved and unresolved, a key take-away with any ^{13}C NMR analysis is the total peak count. For this example, all 20 peaks, both resolved and unresolved, are represented.
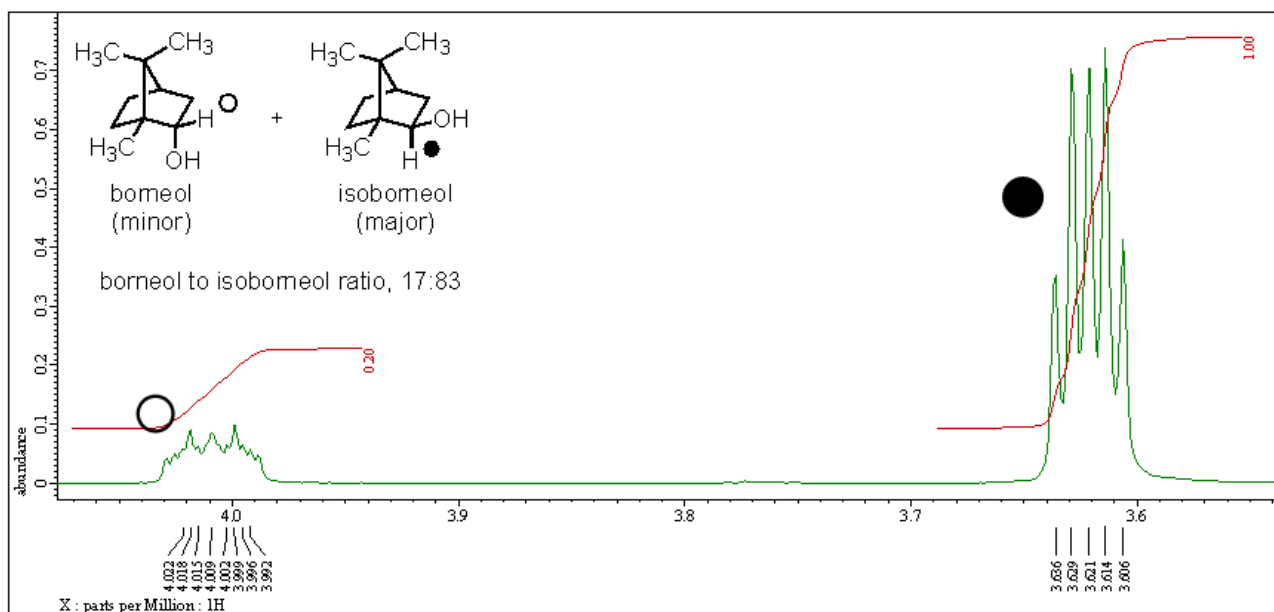

Figure 7. Expanded ^{1}H NMR spectrum of crude reaction mixture of borneol and isoborneol
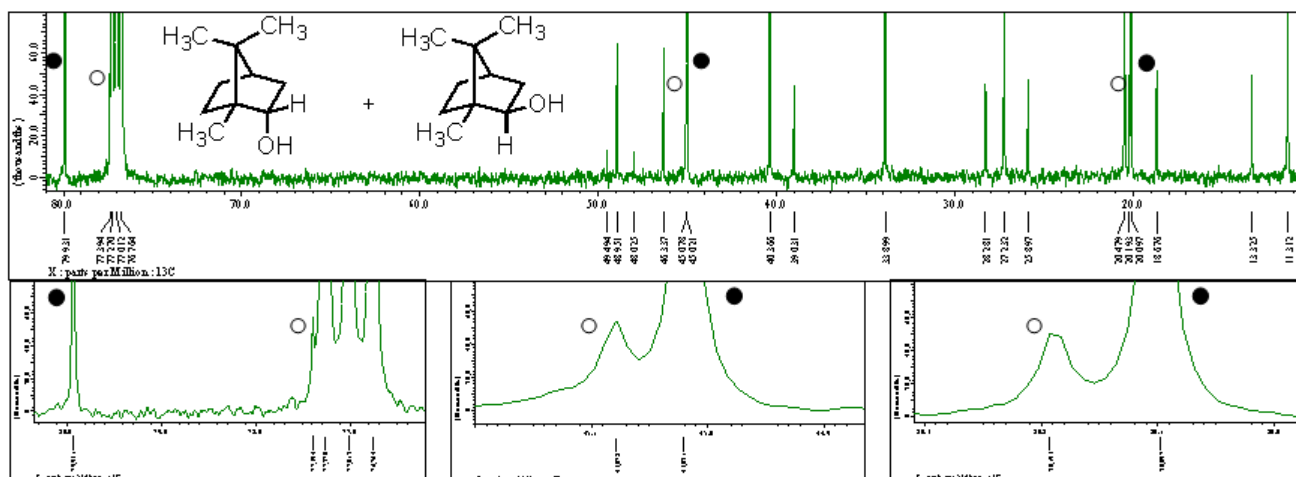

Figure 8. Full and expanded regions of ^{13}C NMR spectrum of crude reaction mixture of borneol and isoborneol

If wanting to delve deeper with this example, at least three options exist with major/minor isomer identification. As the ^{1}H NMR data indicates a heavily favored major to minor ratio, the ^{13}C NMR spectrum can be used qualitatively when assigning chemical shifts of both major and minor isomers using peak intensities alone. While we do not ask students to chromatographically separate the isomers because of time constraints, they can be separated and is a second option with isomeric identification. The third and perhaps the one offering the highest return especially when factoring time constraints is use of NMR band deconvolution on the crude sample. Figure 9 below presents the deconvoluted data using the same three expanded regions shown in Figure 8. For each expanded region, the isomeric compositions are in agreement when compared to the quantitative data obtained using the ^{1}H NMR integrals.

As was the situation in the prior case study, we modified the reaction conditions in an effort to probe the viability of the NMR band deconvolution methodology [22]. The goal was to confirm differences in overall isomeric composition using just ^{1}H and ^{13}C NMR data. We were pleased to find that the methodology was viable as the quantitative data obtained from the crude reaction mixtures when deconvoluting the same expanded regions illustrated in Figure 9 were indeed rapid, easy, and matching that obtained when examining the ^{1}H NMR data. Albeit the data only dropped by 5% and could be within experimental error, the modified reaction conditions using wet alumina as an additive confirmed to us a second opportunity to examine crude reaction mixtures of isomers and by doing so, obtain data of isomeric composition thus adding significant value to the laboratory experience.

And finally, while the extent of coalescence in the aliphatic region removed the ability to apply the NMR band deconvolution methodology using the ^{1}H NMR spectrum, we were with this example able to obtain quantitative data using the resolved chemical shifts of the three methyl groups of both the minor and major isomers.
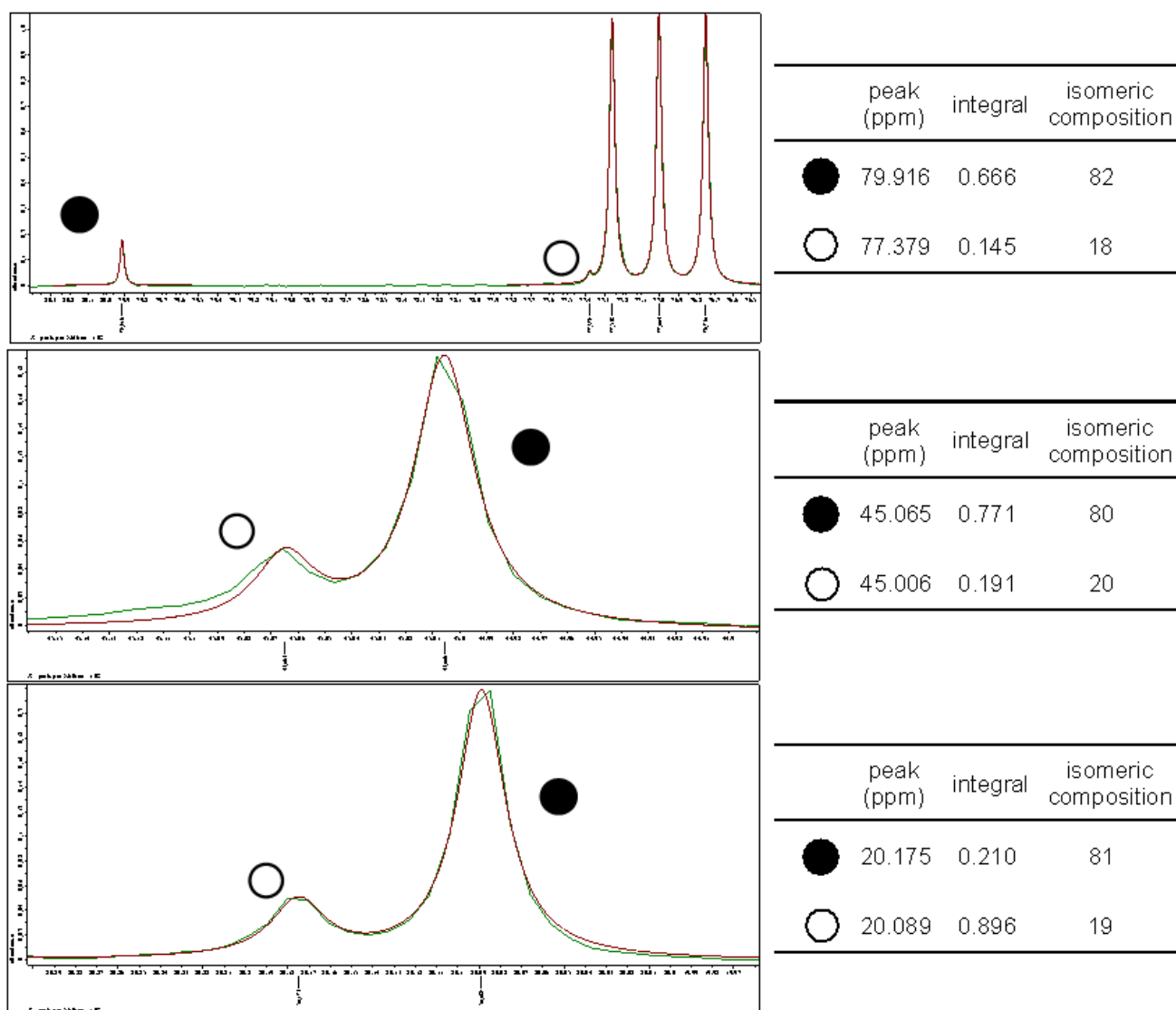

peak (ppm)	integral	isomeric composition
● 79.916	0.666	82
○ 77.379	0.145	18

peak (ppm)	integral	isomeric composition
● 45.065	0.771	80
○ 45.006	0.191	20

peak (ppm)	integral	isomeric composition
● 20.175	0.210	81
○ 20.089	0.896	19

Figure 9. NMR band deconvolutions using expanded regions of the crude spectrum consisting of an isomeric mixture of borneol (unfilled) and isoborneol (filled)

5.3. Case Study: Peak Assignment Confirmation

The third and final case study focused on the identification of the major and minor isomers as part of a mixture where the separation using standard chromatographic techniques was not successful and the resulting isomeric composition was not heavily favoring one over the other allowing for a clear qualitative discernment. While this case study is most likely beyond the scope of a second-year undergraduate experience, it offers an ideal opportunity for those as part of either a special topics or upper level inquiry-based organic laboratory/lecture experience having as the goal the proper use of spectral data in structural identification.

Scheme 4 illustrates the formation of 1-hydroxy-2-methyl-1-phenylpentane from benzaldehyde and 2-pentylmagnesium bromide [23]. This reaction, the Grignard reaction, represents one of thousands where an alkyl magnesium halide has shown great utility in the formation of carbon-carbon bonds which in this case is the formation of a carbon-carbon bond as part of a secondary alcohol. Both substrate and reagent were specifically used because of the anticipated low facial selectivity and opportunity of isomeric confirmation using the NMR band deconvolution methodology.
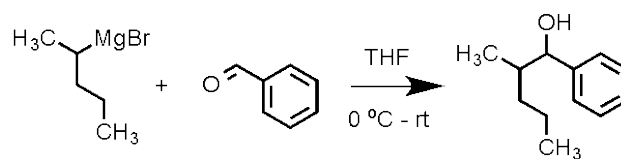
Scheme 4.

Upon workup of the crude reaction mixture or isolation of the secondary alcohol after column chromatography, we noted a ratio of 53:47 using the integrals from the diagnostic peaks of 4.53 ppm and 4.43 ppm of the respective erythro/threo isomers (Figure 10) and ratios ranging from 53:47 to 51:49 using the integrals obtained from the deconvoluted peaks of specifically paired chemical shifts within the doublets and triplets of those below 1.00 ppm (Figure 10 and Figure 11).

After confirming accuracy with each of the coupling constants, the marked peaks of the third upfield peak of the respective triplets, the middle peaks of the respective triplets, or the upfield peak of the respective doublets, we were pleased to see how this methodology continues to illustrate one of the many strengths where peak assignments can be confirmed using isomeric mixtures.
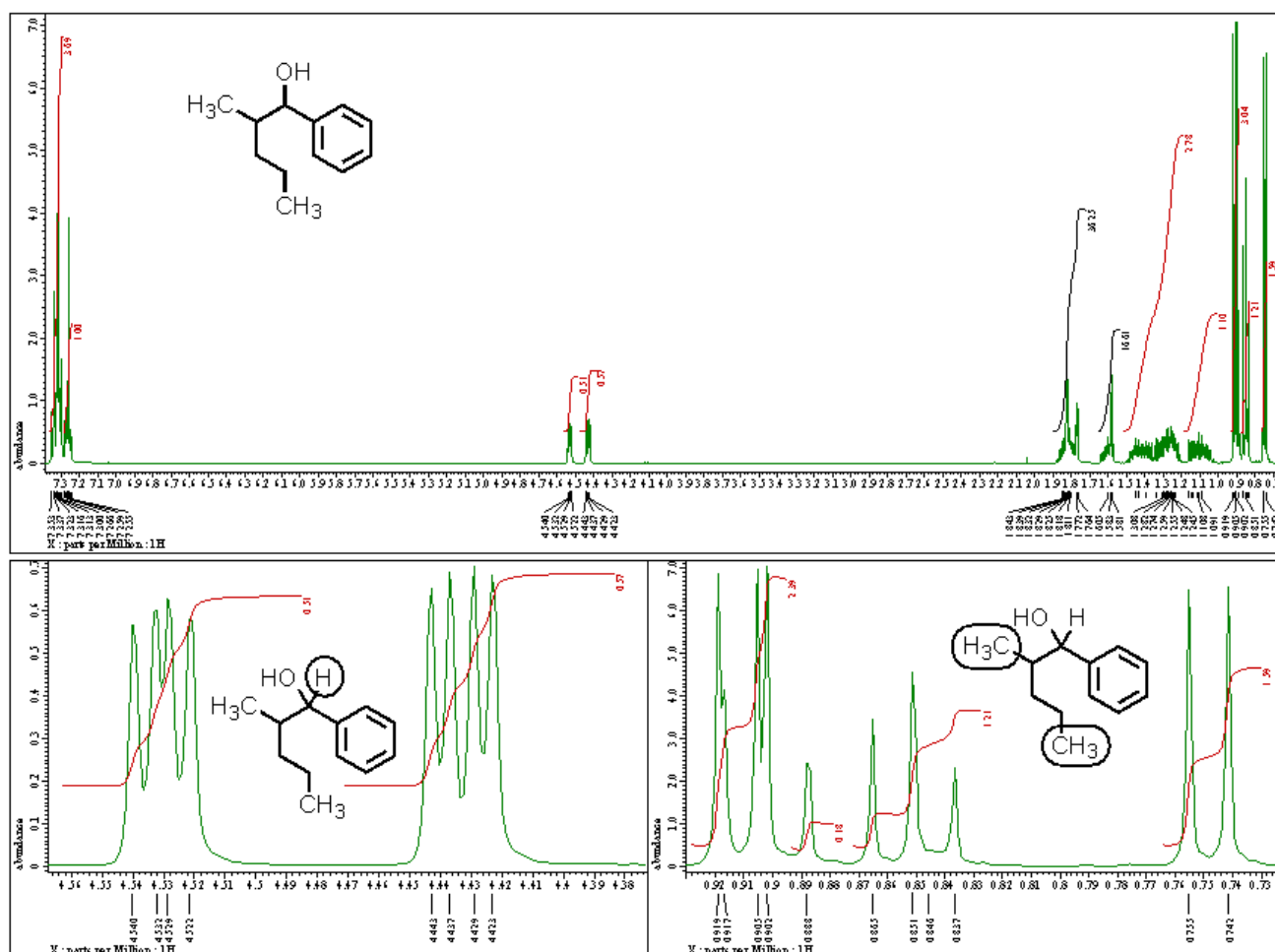
Figure 10. Full and expanded regions of erythro/threo isomers of 1-hydroxy-2-methyl-1-phenylpentane
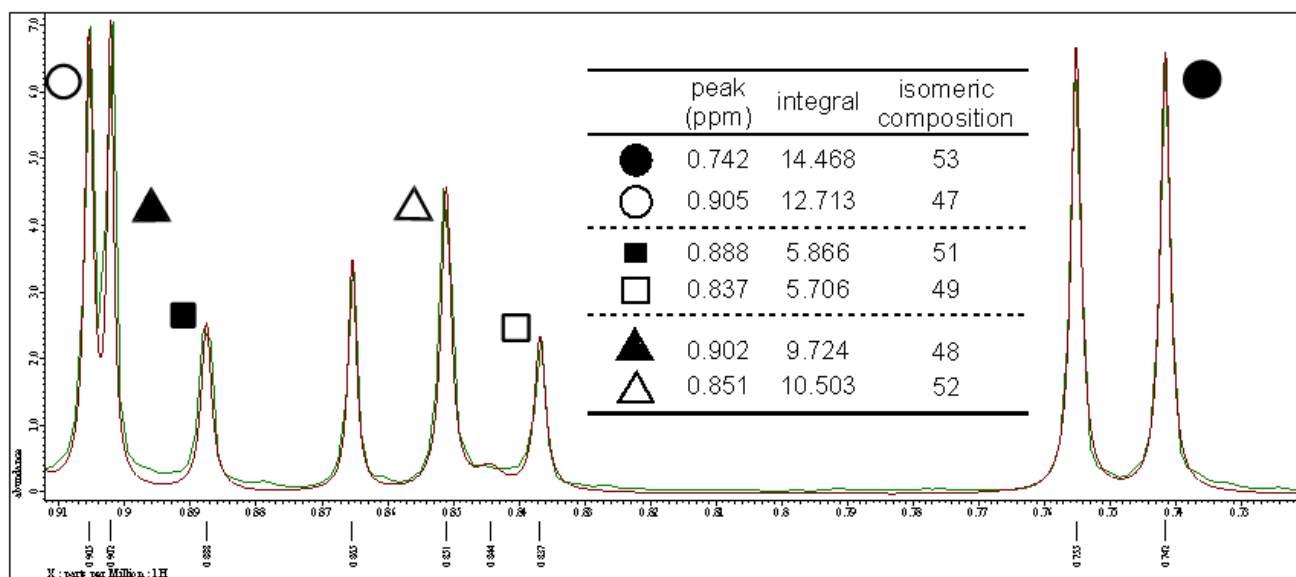
Figure 11. NMR band deconvolutions of an isomeric mixture of major (filled) and minor (unfilled)

The methodology is not without limitations as noted when assessing the isomeric composition of the upfield peak of the doublet at 0.905 ppm (unfilled circle) and middle peak of the triplet at 0.902 ppm (filled triangle). These two peaks are far from resolved and as such, the overlapping signals do not offer clear evidence of isomeric composition. Even with overlapping signals, the opportunity to utilize this methodology to examine isomeric composition exists. Furthermore, as this case study has an isomeric composition of nearly 1:1 and without the opportunity to confirm isomeric composition with the two overlapping peaks around 0.90 ppm (Figure 11), the aromatic region and the region between 1.0 - 1.9 ppm of the ^{1}H NMR spectrum in Figure 10, we were pleased to see confirmation using NMR band deconvolution of both the erythro and threo isomers (Table 3). Furthermore and perhaps most interesting was that the difference in the ratios of the deconvoluted peaks from the non-deconvoluted peaks, specifically those from the hydroxy methine, were nearly identical.

Table 3. Peak assignments

entry	peak assignment	splitting pattern	peak (ppm)	
			major	minor
1	CH at C_1	dd	4.43	4.53
2	CH_3 at C_2	d	0.75	0.91
3	terminal CH_3 (C_5)	t	0.90	0.85

6. Conclusions

The opportunity to obtain quantitative data from ^{1}H and ^{13}C NMR spectra consisting of unresolved peaks, low signal to noise, and spurious peaks was illustrated in three case studies when utilizing NMR band deconvolutions.

Given recent hardware and software advancements as well as the presence of high field spectrometers in academic programs at every level, the methodology can be applied to any organic chemistry laboratory curriculum and perhaps more importantly any synthetic organic research program involving undergraduate researchers. The three case studies are intended to illustrate the application of the methodology. Significant with the approach toward ^{1}H and ^{13}C NMR data analysis and illustrated in each case study is that quantitative data can be obtained. Furthermore, the examples provided documented instances where the chemical shifts of the peak(s) of interest stemmed from differences which ranged from as low as one tenth of a ppm to hundredths of a ppm. Also significant was the ability to confirm isomeric composition in systems of low stereoselectivity which arguably is the strength of this approach when data analysis is crucial to the planned activity. Other benefits include how easy one can obtain accurate and reliable quantitative data (relative not absolute (qNMR)). In most instances, the data stems from crude reaction mixtures making the method rapid and most welcomed for projects focusing on method development. Shifting to more quantitative rather than qualitative analyses of the ^{1}H and ^{13}C NMR spectra, analyses of this type truly allow for the experimentalist to develop critical thinking skills. When used in the instructional lab setting, the opportunity to reinforce key concepts presented as early as the sophomore level exists. Tangential metrics include delving deeper into intermediate/product stability and how they dictate isomeric composition. Thus the challenge, especially for those teaching sophomore level instructional labs, is placing an emphasis on spectral interpretation having as the goal one obtaining and then commenting on quantitative data obtained using the resources available.

Competing Financial Interests

The authors declare no competing financial interests.

References

[1] Bharti, S. K., Roy, R., "Quantitative ^{1}H NMR Spectroscopy," *TrAC*, 35, 5-26, May 2012.

[2] Simmler, C., Napolitano, J. G., McAlpine, J. B., Chen, S.-N., Pauli, G. F., "Universal Quantitative NMR Analysis of Complex Natural Samples," *Current Opinion in Biotechnology*, 25, 51-59, Feb. 2014.

[3] Huang, S. R., Palmer, P. T., "Improving Student Understanding of Qualitative and Quantitative Analysis via GC/MS using a Rapid SPME-Based Method for Determination of Trihalomethanes in Drinking Water," *J. Chem. Educ.*, 94 (8), 1129-1132, Jul. 2017.

[4] Fry, C. G., Hofstetter, H., Bowman, M. D., "A First Laboratory Utilizing NMR for Undergraduate Education: Characterization of Edible Fats and Oils by Quantitative ^{13}C NMR," *J. Chem. Educ.*, 94 (9), 1319-1323, Jun. 2017.

[5] Anderson, S. L., Rovnyak, D., Strein, T. G., "Identification of Edible Oils by Principal Component Analysis of ^{1}H NMR Spectra," *J. Chem. Educ.*, 94 (9), 1377-1382, Jul. 2017.

[6] Pauli, G. F., Gödecke, T., Jaki, B. U., Lankin, D. C., "Quantitative ^{1}H NMR. Development and Potential of an Analytical Method: An Update," *J. Nat. Prod.*, 75 (4), 834-851, Apr. 2012.

[7] Zivkovic, A., Bandolik, J. J., Skerhut, A. J., Coesfeld, C., Zivkovic, N., Raos, M., Stark, H., "Introducing Students to NMR Methods Using Low-Field ^{1}H NMR Spectroscopy to Determine the Structure and the Identity of Natural Amino Acids," *J. Chem. Educ.*, 94 (1), 115-120, Dec. 2017.

[8] Hilty, C., Bowen, S., "An NMR Experiment Based on Off-the-Shelf Digital Data-Acquisition Equipment," *J. Chem. Educ.*, 87 (7), 747-749, May 2010.

[9] Harrell, M. L., Bergbreiter, D. E., "Using ^{1}H NMR Spectra of Polymers and Polymer Products to Illustrate Concepts in Organic Chemistry," *J. Chem. Educ.*, 94 (11), 1668-1673, May 2017.

[10] Collard, D. M., Jones, A. G., Kriegel, R. M., "Synthesis and Spectroscopic Analysis of a Cyclic Acetal: A Dehydration Performed in Aqueous Solution," *J. Chem. Educ.*, 78 (1), 70-72, Jan. 2001.

[11] Pavia, D. L., Lampman, G. M., Kriz, G. S., Engel, R. G., *A Microscale Approach to Organic Laboratory Techniques*, 5th ed., Cengage: Boston, 2013.

[12] Organic Chemistry Instructional Laboratory schedules. https://www.southalabama.edu/colleges/artsandsci/chemistry/laboratories.html [accessed 7 Feb. 2022].

[13] Saba, S., Corozo-Morales, A., "Synthesis and NMR-Spectral Analysis of Achiral *O,O*- and *N,N*-Acetals: Anisochronous pro-*R* and pro-*S* Ligands in NMR Spectra," *J. Chem. Educ.*, 96 (2), 354-359, Dec. 2019.

[14] Saba, S., Ciaccio, J. A., Espinal, J., Aman, C. E., "Synthesis and NMR Spectral Analysis of Amine Heterocycles: The Effect of Asymmetry on the ^{1}H and ^{13}C NMR Spectra of N,O-Acetals," *J. Chem. Educ.*, 84 (6), 1011-1013, Jun. 2007.

[15] Mostad, S. B., Glasfeld, A., "Using High Field NMR to Determine Dehydrogenase Stereospecificity with Respect to NADH: An Undergraduate biochemistry lab," *J. Chem. Educ.*, 70 (6), 504-506, Jun. 1993.

[16] Richardson, D. P., Wilson, W., Mattson, R. J., Powers, D. M., Dolan, B. T., "Discovering Stereoselectivity: Synthesis of Exo- and Endobrevicomin Using a Tunable Hydride Reduction: A Program of Organic Synthesis Experiments for Advanced Undergraduate Students," *J. Chem. Educ.*, 68 (11), 951-955, Nov. 1991.

[17] Mihaleva, V. V., Korhonen, S.-P., van Duynhoven, J., Niemitz, M., Vervoort, J., Jacobs, D. M., "Automated Quantum Mechanical Total Line Shape Fitting Model for Quantitative NMR-Based Profiling of Human Serum Metabolites," *Anal. Bioanal. Chem.*, 406 (13), 3091-3102, May 2014.

[18] Fakayode, S. O., "Purity Analysis of the Pharmaceuticals Naproxen and Propranolol: A Guided-Inquiry Laboratory Experiment in the Analytical Chemistry Laboratory," *J. Chem. Educ.*, 92 (1), 157-162, Aug. 2014.

[19] For detailed instructions in the area of band deconvolutions, see: Delta™ NMR Data Processing Software. https://www.jeolusa.com/PRODUCTS/Nuclear-Magnetic-Resonance/Delta-NMR-Software [accessed 7 February 2022].

[20] University of South Alabama Department of Chemistry Safety Rules. https://www.southalabama.edu/colleges/artsandsci/chemistry/resources/department_safety_rules_a.pdf [accessed 7 February 2022]

[21] Letson, J. N., "Synthesis of Trimedlure from *trans*-6-Methyl-3-cyclohexene-1-carboxylic acid," BS Honors Thesis, University of South Alabama, Mobile, AL, 2018.

[22] Yakabe, S., Hirano, M., Morimoto, T., "Alumina-assisted Reduction of Carbonyl Compounds with Sodium Borohydride in Hexane," *Can. J. Chem.*, 76 (12), 1916-1921, Dec. 1998.

[23] Taken from an adaptation on the preparation of 3-hydroxy-4-methylheptane from propanal, see: Mayo, D. W., Pike, R. M., Forbes, D. C., *Microscale Organic Laboratory with Multistep and Multiscale Syntheses*, 6th ed., Wiley: New York, 2015.