

STRUCTURAL ELUCIDATION OF UNKNOWN: A SPECTROSCOPIC INVESTIGATION WITH AN EMPHASIS ON 1D AND 2D ^1H NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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Abstract. Nuclear Magnetic Resonance (NMR) spectroscopy is a widely used technique for structural elucidation. It is often used in conjunction with other complimentary analytical techniques such as infra-red (IR) and mass spectrometry (MS) to completely assign the chemical structure of molecules. For undergraduate students, gaining familiarity with NMR is often difficult as they rarely have the opportunity to collect this data for themselves; often they are just provided with spectra to interpret. We have developed an experiment that tasks students with obtaining 1D and 2D ^1H NMR data of two unknowns, and then using $^{13}\text{C}\{^1\text{H}\}$ NMR, CHN elemental microanalysis, MS and IR data, to elucidate fully their two structures. The experiment provides students with an experiential learning opportunity for ^1H NMR whilst also developing and strengthening their structural elucidation skills.

Keywords: second-year undergraduate; organic chemistry; hands-on learning; problem solving; NMR spectroscopy

Introduction

NMR (Nuclear Magnetic Resonance) is a powerful analytical technique; it enables the connectivity of atoms to be deduced and, by using this information, elucidate the structure of a compound. It is an integral technique that is utilised in research and design worldwide, particularly within the pharmaceutical industries for analysis of new drug compounds, but also in materials science, food analysis and clinical studies (Gerothanassis et al., 2002). Despite the inherent sensitivity issue associated with NMR, which is more evident at low magnetic field strengths, several companies have now developed benchtop NMR devices which are cheaper than their associated high-field, superconducting counterparts. A streamlined approach to the collection of NMR data has been taken by these companies, resulting in the

devices being suitable for enabling students to collect NMR data with minimal input from instructors (Yearty et al., 2017; Zivkovic et al., 2017a; 2017b).

Typically, students are not exposed to NMR spectroscopy practically as much as other spectroscopic techniques, such as IR, even though the positive benefits of the inclusion of high-field FT-NMR in the undergraduate curriculum has been noted (Ball & Miller, 2002). Some laboratory facilitators have designed experiments to introduce high-school students to NMR spectroscopy using low-field NMR to increase exposure to the technique prior to entering tertiary education (Bonjour et al., 2015). NMR sites, such as the one at Trinity University which comprises spectral data of student samples from community colleges, have been utilised to provide students with the unprocessed free induction decay (FID) so that they may process the data to generate relevant spectra (Mills & Shanklin, 2011). However, this does not provide students with hands-on experience of data collection. Students can often have a perceived complexity of the technique, in that NMR is typically foreseen as a “black box” technique with students “simply click[ing] on a mouse button and collect[ing] a spectrum without fully understanding the mechanics of the instrument” (Lorigan et al., 2001). Although theoretical knowledge of NMR is important, actually conducting an NMR-based experiment helps improve student understanding as evidenced by post-lab qualitative student self-assessment and identical quantitative pre- and post-lab tests (Wagner et al., 2013).

NMR analysis can be used alongside other analytical techniques, such as thin layer chromatography, to monitor organic transformations. For example, the hydrogenation of curcumin using a hydrogenation flow reactor has been described (Wagner et al., 2013). Part of the practical experiment involved assigning the ^1H NMR spectra of curcumin and its hydrogenated analogue, tetrahydrocurcumin. Both products have many structural features to demonstrate ^1H NMR shifts, and the nuclei possess spin-spin couplings to help further elucidate their structures. Furthermore complex pulse sequences, such as DEPT (Distortionless Enhancement by Polarisation Transfer), have been employed for the identification of unknown alcohols in a second-year undergraduate course (Chamberlain, 2013).

Structural elucidation is an important skill that students continually develop throughout their undergraduate degree and beyond, mainly through enquiry-based learning (EBL). The use of EBL has been reported to increase the confidence of first-year chemistry students learning spectroscopy (Lucas & Rowley, 2011). Opportunities to apply these skills and develop them further must be provided through an undergraduate curriculum (Carroll et al., 2012; Cerrada & Laguna, 2005; Dávila & Widener, 2002; Grant & Latimer, 2003; Nazri et al., 2012; Walsh et al., 2012). Herein, we describe a practical experiment for second-year undergraduate students that has a focus on ^1H NMR, but also utilises $^{13}\text{C}\{^1\text{H}\}$ NMR, CHN elemental microanalysis, IR and MS data to elucidate the two unknowns. The main pedagogical goals of this experiment are to: (i) enable students to make links between NMR the-

ory and the “real world” collection of NMR data, analysis and interpretation from a practical perspective through experiential learning; (ii) demonstrate to students the power of NMR in the structural elucidation of organic compounds through the collection of 1D and 2D NMR data; (iii) demonstrate to students that analytical techniques should not be used in isolation but in tandem with one another in order to successfully elucidate the structures of molecules

General experiment and discussion

This experiment¹⁾ is intended to act as a support laboratory session for students as part of a core organic module in which NMR and other spectroscopic techniques are taught alongside organic chemistry that has as one of its many focuses the formation and reactions of enolates. Thus, the laboratory session is designed to link these two elements together so that students utilise knowledge from different parts of their course in order to complete the assessment. The session is timetabled for two hours in groups of ten students. The number of students in the group may be increased if more than one spectrometer is available.

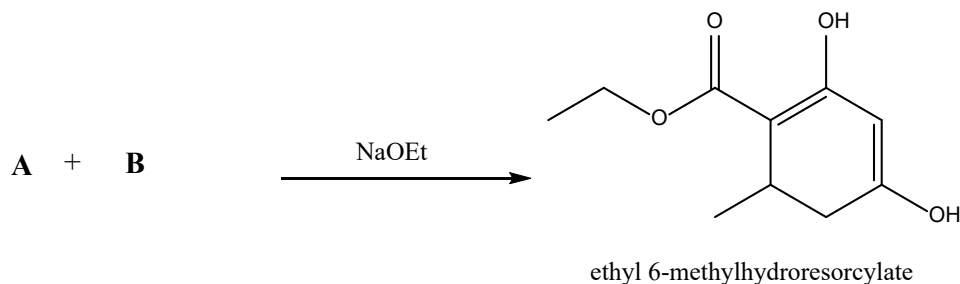


Figure 1. Reaction scheme for the formation of ethyl 6-methylhydroresorcyate. Students are tasked with elucidating the structures of A and B and a mechanism for how they subsequently react with one another to form ethyl 6-methylhydroresorcyate

The experiment is focused on the identification of two unknowns, ethyl crotonate (unknown A) and ethyl acetoacetate (unknown B) respectively; students are told that they react with one another in the presence of sodium ethoxide to produce the product, ethyl 6-methylhydroresorcyate, as shown in Fig. 1. Ethyl 6-methylhydroresorcyate is a precursor in the synthesis of (\pm)-daurichromenic acid (Fig. 2) which has been shown to have potent anti-HIV activity ($EC_{50} = 0.00567 \mu\text{g/ml}$) (Hu et al., 2004). Furthermore, ethyl 6-methylhydroresorcyate is a necessary material in the biosynthesis of PD 116198, a benz[a]anthraquinone antibiotic, produced by *Streptomyces phaeochromogenes* WP 3688 (Gould & Cheng, 1993). As part of the write up, students are asked to suggest a reaction mechanism for the formation of

ethyl 6-methylhydroresorcyate. The mechanism consists of a Michael addition followed by a Claisen condensation, and thus draws upon the knowledge that students will be developing at this stage in their undergraduate course.

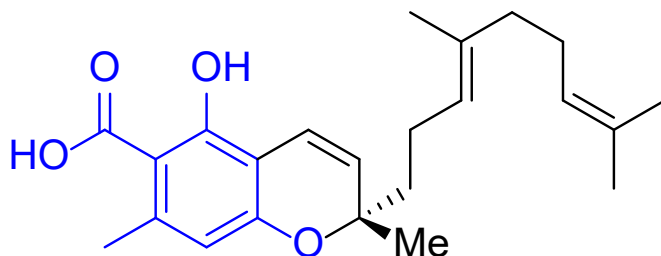


Figure 2. Chemical structure of daurichromenic acid: the part of the structure highlighted in blue is derived from ethyl 6-methylhydroresorcyate

Students are provided with CHN elemental microanalysis, IR spectra, mass spectra (electron impact), DEPT and $^{13}\text{C}\{^1\text{H}\}$ NMR spectra to help in their elucidation of the two unknown molecules. From the elemental microanalysis data and mass spectra provided, students apprehend that the two compounds only differ by a single oxygen atom (unknown A and B have molecular formulae of $\text{C}_6\text{H}_{10}\text{O}_2$ and $\text{C}_6\text{H}_{10}\text{O}_3$ respectively). Students should be encouraged to think about functional groups that contain oxygen e.g. ketones, esters, alcohols and use the IR spectra to collaborate or disprove their hypotheses. Key peaks in the IR spectra are detected at 1721.5 cm^{-1} ($\text{C}=\text{O}$) and 1661.9 cm^{-1} ($\text{C}=\text{C}$, alkene) for unknown A and a split peak at 1717.8 cm^{-1} (two different $\text{C}=\text{O}$) for unknown B. As esters easily fragment in the mass spectrometer when electron impact is employed, this is prudent to bring to the student's attention as well; both unknowns have a weak mass ion in their corresponding mass spectra that reflects this. The appearance of a peak at $\delta 144.6$ in the $^{13}\text{C}\{^1\text{H}\}$ NMR for unknown A and at $\delta 199.7$ and 166.4 for unknown B, all of which are not present in the DEPT, reinforce observations in the IR spectra that quaternary, very de-shielded, carbon nuclei are present.

Students are required to collect the ^1H NMR spectra and COSY data to complete the necessary characterisation data in order to fully elucidate the structures of unknowns A and B. These data highlight the significant structural differences between the two compounds. A benchtop NMR spectrometer operating at 60 MHz is more than adequate for data collection (an Oxford Instruments' Pulsar equipped with a $^1\text{H} / ^{19}\text{F}$ probe was utilised for this work). The facilitator of the session gives students an introduction detailing the purpose of the experiment and an overview of the benchtop NMR device. Safety concerns associated with working in the vicinity of a powerful magnet are also raised e.g. precautions should be taken for students

possessing pacemakers or metal-implants. Students were provided with clear user guides for the collection and processing of ^1H NMR data.¹⁾ As students will have unrestricted access to the benchtop NMR in the third year of their studies at Manchester Metropolitan University, this provides them with an ample introduction to the instrument and allows them to gain experience using it.

The collection of the ^1H - ^1H COSY (Correlation Spectroscopy) data is run as a group exercise because of the time that is involved in collecting them (~30 min). A building-up approach to 2D NMR spectroscopy using high-field instrumentation has recently been described for second-year undergraduate students with a focus on COSY, HMBC (heteronuclear multiple bond coherence) and HSQC (heteronuclear single quantum coherence) (Anderson-Wile, 2016); crucially this means that the techniques can be revisited in advanced undergraduate courses. Although only COSY is focused on herein, this still provides ample introduction to 2D NMR spectroscopy. Whilst the ^1H - ^1H COSY NMR spectrum is being acquired, students are tasked with sample preparation. The ^1H - ^1H COSY NMR spectrum of ethyl crotonate, unknown A, is shown in Fig. 2; it clearly shows the expected couplings between the CH_2 and CH_3 of the ethyl group, as well as the couplings between the alkenic protons (a mixture of $^3J_{\text{HH}}$ and $^4J_{\text{HH}}$ couplings) and the terminal methyl group. The latter couplings are more complex than the former and as such, the ^1H - ^1H COSY NMR spectrum aids the students in elucidating this structural component of the molecule. It can also be useful in deciphering why the complex coupling patterns in the corresponding ^1H NMR spectrum arise. Students collect the corresponding 1D ^1H NMR spectra for unknowns A or B themselves within their pairs and fully process the data once collected. Spectra were collected using 2 M samples, thus entailing that they are readily collected in one scan and provide adequate signal-to-noise for processing purposes. Data processing can be done on an individual basis, or as seemed commonplace, students would peer support one another through the process using the user guides provided to guide them. Data was processed using the MNOVA software package.

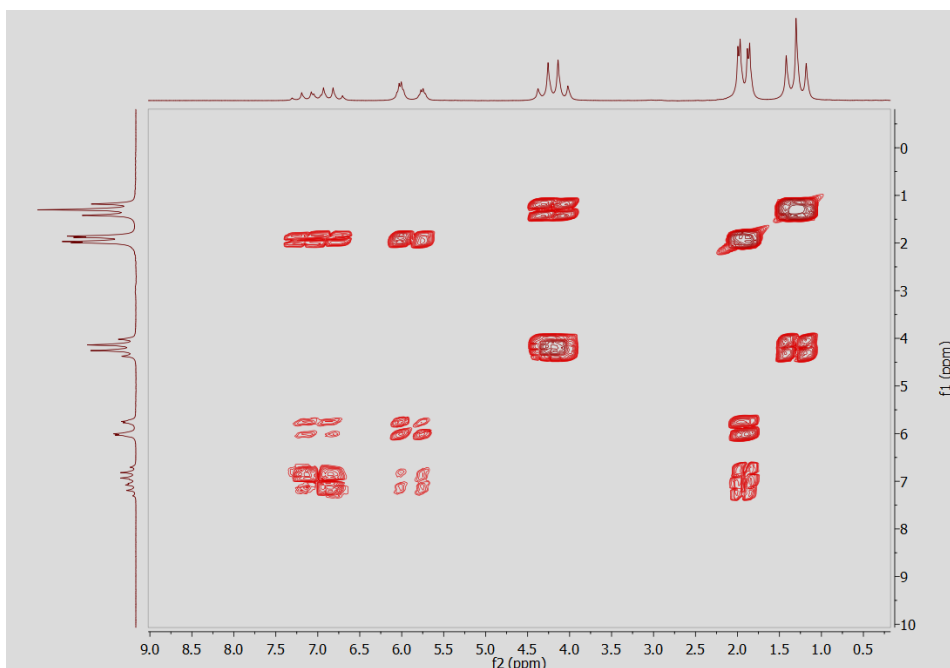


Figure 3. ^1H - ^1H COSY NMR spectrum of ethyl crotonate (compound A) in acetone- D_6

The alkenic moiety in ethyl crotonate gives rise to interesting multiplets; even when collected on a 60 MHz instrument, these are sufficiently resolved to deduce the couplings within each multiplet. Coupled with the integral ratios, it is therefore possible for students to not only deduce the chemical environment which gives rise to the signals observed in the ^1H NMR spectrum (Fig. 3) but to also deduce the geometrical isomerism of the alkene moiety by deducing the appropriate coupling constants. This is an aspect of the experiment that is pressed upon students; other spectroscopic techniques such as IR and GC-MS will only indicate the presence of an alkene or the molar mass of the material but will not provide information about how other groups attached to it are arranged in space. The alkenic carbon signals are observed at δ 144.6 and 124.0 in the $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum and are both positive in the DEPT. Couplings can also be deduced for the methylene and methyl groups of the ethyl group – these show identical coupling constants and thus reinforce the observation of the corresponding cross peak in the ^1H - ^1H COSY. They are observed at δ 60.6 and 14.9 in the $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum; the latter is positive in the DEPT whereas the former is negative, thus reinforcing its assignment as a methylene carbon.

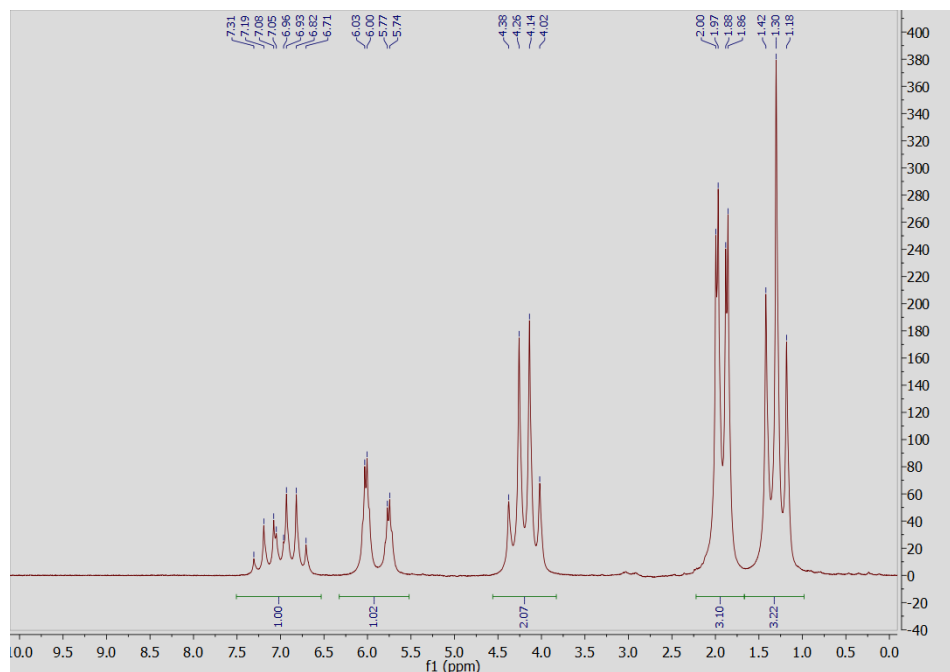


Figure 4. ^1H NMR spectrum of compound A collected in acetone- D_6

The ^1H NMR spectrum of ethyl acetoacetate, unknown B, possesses enol-keto tautomerism along with the expected peaks. This ties in very well with the enolate chemistry that the students learn during the second year of their studies. When students are collecting this spectrum, it is worth having a demonstrator on hand to point out the presence of additional peaks (marked with an asterisk in Fig. 4) and to facilitate useful discussion regarding the possibilities as to how they may arise (Drexler & Field, 1976). Students find this useful as it helps them to visualise the differences in chemical structure between the enol and keto forms based on the chemical shift data that is contained within the ^1H NMR.

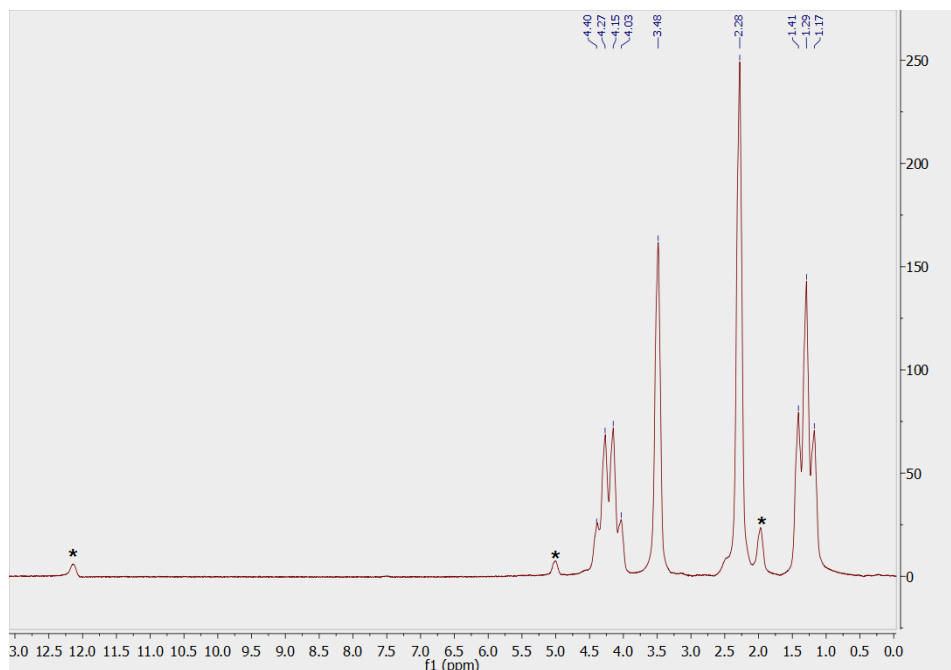


Figure 5. ¹H NMR spectrum of compound B collected in Chloroform-D. Peaks marked with an asterisk belong to the enol form

The four peaks of the predominant keto-form suggest a less coupled structure compared to that of ethyl crotonate. In fact, the ¹H-¹H COSY spectrum¹⁾ only shows that two proton environments couple. The presence of two singlets in the ¹H NMR spectrum should be impressed upon the students to get them focused on to how this could occur, and what functional groups may lead them to be deshielded to differing extents. Furthermore, the characteristic coupling of an ethyl group is present (peaks at δ 4.21 and 1.29) and these could be used to compare and contrast against those observed in ethyl crotonate. The ¹³C{¹H} NMR signals of the ethyl moiety will also be of use (δ 60.2 and 13.1) as they are very similar to those of unknown A and, therefore, reinforce structural similarity between the two unknowns.

Results

One second year chemistry cohort ($n = 98$) has completed this assignment. 93% of the students were able to identify molecule A, although only 18% identified it to possess a *trans* alkene. A further 12% of students identified molecule A to possess a *cis* alkene with the remainder not identifying the type of geometrical isomerism present. When the geometrical isomerism was incorrectly identified to be *cis*, this

mainly originated due to the students calculating only one coupling constant for the CH group that is α to the methyl group – this possesses both a 6.6 and 15.6 Hz $^3J_{\text{HH}}$ coupling. The former, when viewed in isolation is suggestive of a *cis* alkene and, therefore, may have steered the students towards this conclusion. 97% of students were able to correctly identify molecule B.

Compared to the previous assignment, which although identical, was purely paper based and consisted of no experiential learning, this assignment resulted in a ca. 10% increase in the average mark obtained by the students who completed it. Of the seven different sections¹⁾ it was the CHN elemental microanalysis section in which students scored the highest (average = 93%). The mass spectrometry, IR spectroscopy, ^1H NMR, $^{13}\text{C}\{^1\text{H}\}$ NMR and mechanistic sections averaged 67 – 75%. The lowest scoring section was where the students were asked to deduce the chemical structures and provide rationale for them. The average for this section was 58% - the lower percentile score was mainly due to students providing more of an overview of the data utilised, rather than picking out key data that helped in the elucidation of the structures.

Qualitative data collected from a group of students ($n = 10$) who helped trial the assignment described herein and who had previously been assessed using the purely paper based assignment stated that “doing a practical as part of the coursework was good and gave me a better understanding” and that it was “a lot more fun to understand a new process”. Having fun and experiencing enjoyment are perceived by adult learners as a motivator to attend classes and learn knowledge and skills (Lucardie, 2014). Students also remarked that the experiment had given them “a wider knowledge of NMR”.

Conclusions

An undergraduate structural elucidation experiment with an emphasis on the collection of 1D and 2D ^1H NMR data is detailed. Students obtained hands-on experience of collecting, processing and interpreting NMR data and used it, in conjunction with other analytical data such as IR and MS data, to deduce the chemical structures of two unknowns and the reaction they undergo to produce ethyl 6-methylhydroresorcylate. Students vastly preferred this to a purely paper based exercise, citing that they could take ownership of the data produced and that they could gain valuable experience of NMR spectroscopy if allowed to collect the data themselves. The pedagogic goals, as outlined in the introduction, were achieved as the experiment enabled students to make links between NMR theory and the collection of “real world” data, and for them to utilise 1D and 2D ^1H NMR spectroscopy, alongside other analytical methods, to elucidate the structure of organic compounds.

One can envisage that this experiment could be part of a larger laboratory class in which students are split in to teams, for example, and asked to collect all the

necessary spectroscopic data to elucidate the structures of the two unknowns. This could be part of a problem-based / enquiry-based learning practical experiment. The experiment could also be further expanded upon by tasking students to synthesise and characterise the product produced (ethyl 6-methylhydroresorcylate) when ethyl crotonate and ethyl acetoacetate react with one another in the presence of base.

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NOTES

1. For the details, contact the corresponding author Dr. R. E. Mewis.

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