Shane Kalani Abbley

Component V Lab Report: Nuclear Magnetic Resonance Spectroscopy

Abstract:

We used NMR in order to elucidate the structures of a fatty acid methyl ester and to better understand the conformers of allantoin in DMSO. We utilized various NMR experiments including: ^{1}H NMR, ^{13}C NMR, DQF-COSY, NOESY, TOCSY, ^{13}C HMBC, ^{13}C HSQC, ^{15}N HMBC, and ^{15}HMBC. Obviously, these experiments produced a lot of redundant data, but on their own they all provide useful information and were relevant in confirming previous assignments. We utilized MestReNova and SpinDynamica in order to simulate coupling constants. We calculated specific dihedral angles from these coupling constants using the Karplus equation in order to create specific conformers of allantoin. We then compared these conformers to previously calculated conformers of allantoin.

Introduction:

For component V, we performed two NMR experiments. Specifically, we studied the NMR spectra of a fatty acid methyl ester to determine and analyze the coupling constants. We also studied various 1D and 2D NMR experiments for allantoin to better understand the relationship between the structure of a molecule and its corresponding spectrum.

Nuclear Magnetic Resonance (NMR) is an experiment that measures the frequency at which ½ spin nuclei achieve resonance based on their chemical environment. This means that at a specific radio frequency pulse a nucleus in a specific chemical environment will emit energy in the form of a free induction decay (FID)[1]. This FID is measured and deconvoluted using a Fourier transform to show the frequencies at which certain nuclei are excited. These spectra are performed most frequently on ^{1}H and ^{13}C, though any ½ spin nuclei exhibit this behavior to some extent. ^{1}H NMR spectra of a molecule would yield a 1D NMR spectrum, where the frequency of the emitted energy is represented on the x-axis and the relative intensity is represented on the y-axis. Typical 1D NMR include ^{1}H NMR and ^{13}C NMR. These experiments are valuable for determining the structure of molecules by providing chemical shift values and splitting patterns. Chemical shift values correspond to the chemical environment of each nuclei and splitting patterns are determined by interactions between coupled nuclei, meaning ½ nuclei that are close to each other. Coupling is more easily observed in 2D NMR experiments, where two 1D spectra are plotted against each other. In some cases two of the same spectra are plotted against each other, as in homonuclear correlation spectroscopy experiments. In other experiments two different spectra are plotted against each other, as in heteronuclear correlation spectroscopy experiments. Coupling can also be represented quantitatively by coupling constants. Coupling constants can be determined by the Karplus Equations. In general, Karplus Equations relate the coupling constants ($^{3}J(\phi)$) between vicinal hydrogens to the values of the dihedral angles (ϕ) between said hydrogens and molecular parameter constants (A,B,C) as such:

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$$^{3}J(\phi) = A\cos^{2}(\phi) + B\cos(\phi) + C^{2}.$$

Allantoin is a molecule of interest due to its metabolic relevance and its frequent commercial use[3]. Allantoin is the end product of urate degradation and thus is an important component of the purine degradation pathway[4]. We utilized 1D NMR and 2D NMR experiments to better understand the structure of allantoin overall and of specific conformers. Specific 2D experiments like COSY and TOCSY provided additional information regarding proton correlations. Other 2D experiments such as the ^{13}C and ^{15}N HMBC and ^{13}C and ^{15}N HSQC experiments gave additional correlation information regarding the hydrogens and their local ^{13}C and ^{15}N nuclei. The NOESY spectra gave spatial information rather than information through bonds. This NOESY spectra in particular should allow for understanding conformers of allantoin based on the distance between specific atoms.

SpinDynamica was utilized in order to perform spin simulations of Allantoin. This allowed for more accurate coupling constant determination values by use of Hamiltonian and/or Liouvillian superoperators[5].

MestReNova was used to visualize the spectra. In particular, we zoomed in to all the spectra provided on the course website in order to observe the splitting patterns, chemical shift values, and integration values. We also used MestReNova in order to deconvolute some of the spectra to better visualize assignments as well as to estimate coupling constants.

Methods:

Parameters for Each Experiment:

Fatty Acid Methyl Ester ^{1}H NMR: The spectrum for this experiment was obtained at 25 ℃ on the Bruker 500 MHz NMR instrument.

Allantoin ^{1}H NMR at 25 ℃: This spectrum was collected with DMSO in the Bruker 600 MHz instrument, with an acquisition time of 4 seconds, a relaxation delay of 6 seconds, with 32 repetitions, and at 25 ℃.

Allantoin ^{1}H NMR at 50 ℃: This spectrum was collected with DMSO in the Bruker 600 MHz instrument, with an acquisition time of 3.85 seconds, a relaxation delay of 6.15 seconds, with 32 repetitions, and at 50 ℃.

Allantoin ^{13}C NMR with ^{1}H NMR Coupling: This spectrum was collected with 50 mg of allantoin in DMSO in the Bruker 600 MHz instrument, with an acquisition time of 3.26 seconds, a relaxation delay of 6.8 seconds, with 1024 repetitions, and a spectral width of 20100 Hz at 25 ℃.

Allantoin ^{13}C NMR with ^{1}H NMR Decoupling: This spectrum was collected with 50 mg of allantoin in DMSO in the Bruker 600 MHz instrument, with an acquisition time of 2.6 seconds, a relaxation delay of 7.4 seconds, with 256 repetitions, and a spectral width of 25125 Hz at 25 ℃.

Allantoin ^{13}C NMR with Inverse ^{1}H NMR Coupling: This spectrum was collected with 50 mg of allantoin in DMSO in the Bruker 600 MHz instrument, with an acquisition time of 3.26

seconds, a relaxation delay of 20 seconds, with 368 repetitions, and a spectral width of 20100 Hz at 25 ℃.
Allantoin DQF-COSY NMR: This spectrum was collected in DMSO in the Bruker 600 MHz instrument, with an acquisition time of 0.30 seconds, a relaxation delay of 1.5 seconds, with 4 repetitions, and a spectral width of 6340.6 Hz at 25 ℃.
Allantoin NOESY NMR: This spectrum was collected in DMSO in the Bruker 600 MHz instrument, with an acquisition time of 0.150 seconds, a mixing time of 0.50 seconds, a relaxation delay of 3.0 seconds, with 4 repetitions, and a spectral width of 6340.6 Hz at 25 ℃.
Allantoin TOCSY NMR: This spectrum was collected in DMSO in the Bruker 600 MHz instrument, with an acquisition time of 0.750 seconds, a mixing time of 0.05 seconds, a relaxation delay of 1.0 seconds, with 4 repetitions, and spectral widths of 6492.5 Hz (F2) and 6504.1 Hz (F1) at 25 ℃.
Allantoin ^{13}C HMBC NMR (500 and 600 MHz Bruker Instrument): This spectrum was collected in DMSO with 1 mg of urea, with an acquisition time of 0.17 seconds, a relaxation delay of 2.5 seconds, with 8 repetitions, with coupling parameters 150 Hz (J1xh) and 7 Hz (Jnxh), and spectral widths of 600.6 Hz (F2) and 26838.6 Hz (F1) at 25 ℃.
Allantoin ^{15}N HSQC NMR: This spectrum was collected in DMSO in the Bruker 600 MHz instrument, with an acquisition time of 0.10 seconds, a coupling parameter (jxh) of 90 Hz, a relaxation delay of 2.0 seconds, with 8 repetitions, and spectral widths of 10319.9 Hz (F2) and 9115.8 Hz (F1) at 25 ℃.
Allantoin ^{15}N HMBC NMR: This spectrum was collected in DMSO in the Bruker 600 MHz instrument, with an acquisition time of 0.49 seconds, with coupling parameters 95 Hz (J1xh) and 12 Hz (Jnxh), a relaxation delay of 1.4 seconds, with 32 repetitions, and spectral widths of 8394.9 Hz (F2) and 14587.9 Hz (F1) at 25 ℃.
Spindynamica was used to model spin dynamics using Hamiltonian and/or Liouvillian superoperators.
Karplus Equations used are from Schmidt and Blümel as described above[2].

Results:

The following data was analyzed according to the provided operations manual.

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Fatty Acid Methyl Ester ^{1}H NMR:

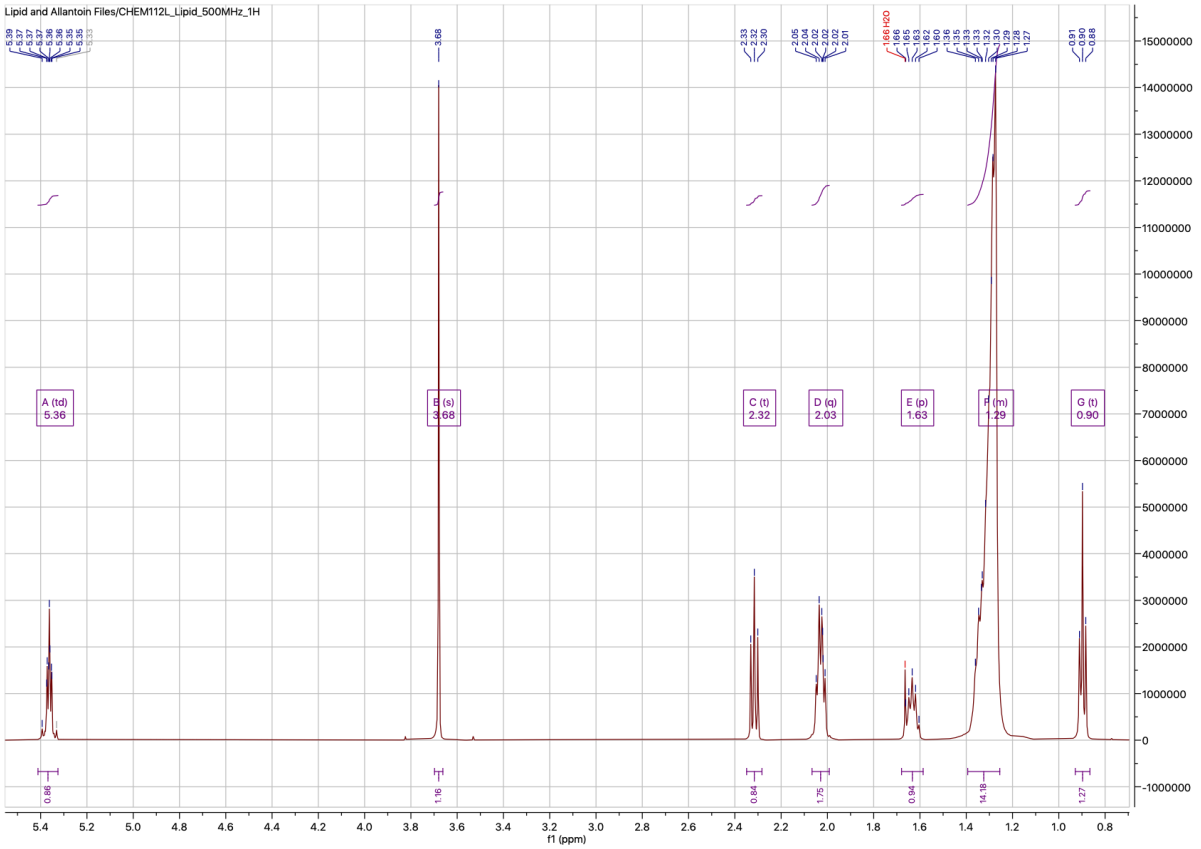

Figure 1: ^{1}H NMR Spectrum of a Fatty Acid Methyl Ester
This figure shows the relevant peaks in the spectra of the provided fatty acid methyl ester.

Name	Chemical Shift (ppm)	Number of Protons	Integration	Coupling Constants	Likely Assignment
A	5.34	2	2.06	2.12, 4.41, 4.27	Vinylic Hydrogens
B	3.66	3	3.00	N/A	Methoxy Hydrogens
C	2.29	2	2.05	7.57, 7.57	Alpha Hydrogens
D	2.01	4	4.01	6.72, 6.72, 7.04	Allylic Hydrogens
E	1.61	2	2.35	7.04, 7.04, 7.33, 7.33	Beta Hydrogens
F	1.27	32	32.26	N/A	Alkyl Chain Hydrogens
G	0.87	3	3.07	6.83, 6.83	Methyl Tail Hydrogens

Figure 2: Analysis of the Spectrum of the Fatty Acid Methyl Ester
This table gives the assignment of the peaks in Figure 1 based on their chemical shift, integration and multiplicity. The coupling constants were determined by MestReNova.

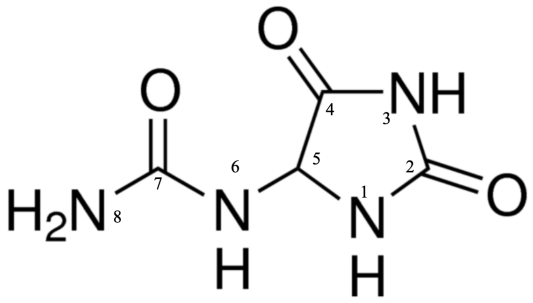

Figure 3: Reference Allantoin Assignments
This depiction of allantoin gives reference assignments for the following figures.

The probability of dihedral angle values were determined by a quantum mechanical energy minimization of allantoin. The most favorable dihedral angle for H1-N1-C5-H5 was about 40°, which corresponds to a coupling constant of about 4.86. The most favorable dihedral angle for H6-N6-C5-H5 was about 250°, which corresponds to a coupling constant of about 0.86.

Allantoin ^{1}H NMR:

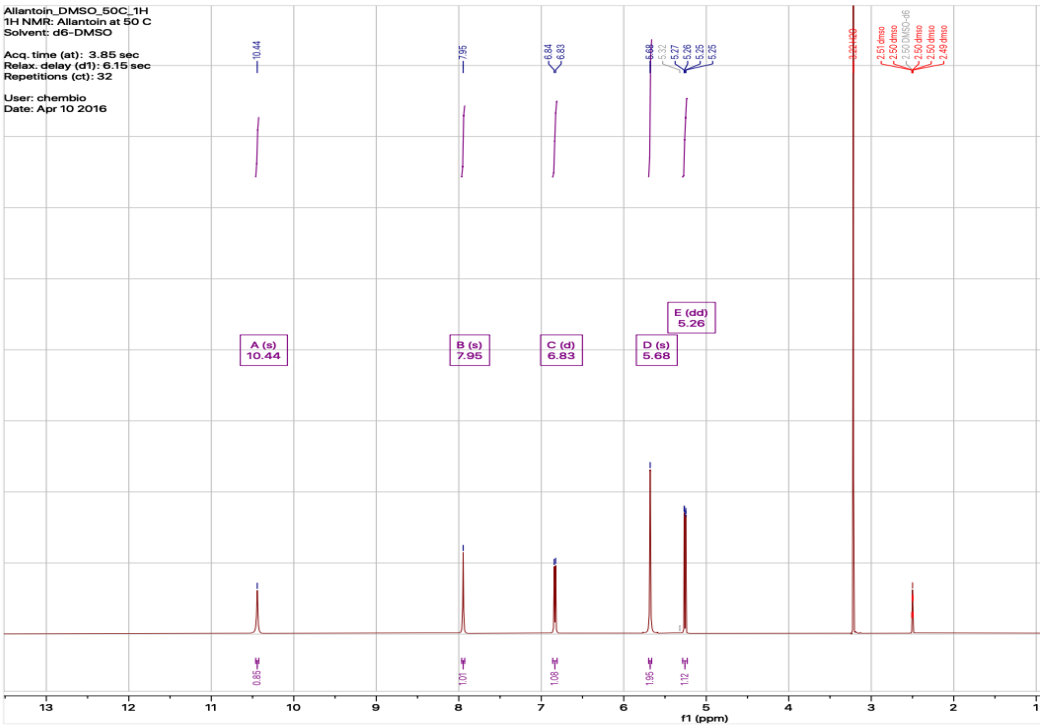

Figure 4: ^{1}H NMR Spectrum of Allantoin in DMSO
This figure shows the relevant proton NMR peaks for allantoin in DMSO.

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Name	Chemical Shift (ppm)	Number of Protons	Integration	Coupling Constants	Assignment
A	10.44	1	0.85	N/A	H3
B	7.95	1	1.01	N/A	H1
C	6.83	1	1.08	8.20	H6
D	5.68	2	1.95	N/A	H8(a) and H8(b)
E	5.26	1	1.12	1.42, 8.23	H5

Figure 5: Assignment of ^{1}H NMR Spectrum for Allantoin in DMSO
This table gives assignments for the proton NMR of allantoin in DMSO using chemical shift and estimated coupling constants from MestReNova.

Allantoin ^{13}C NMR:

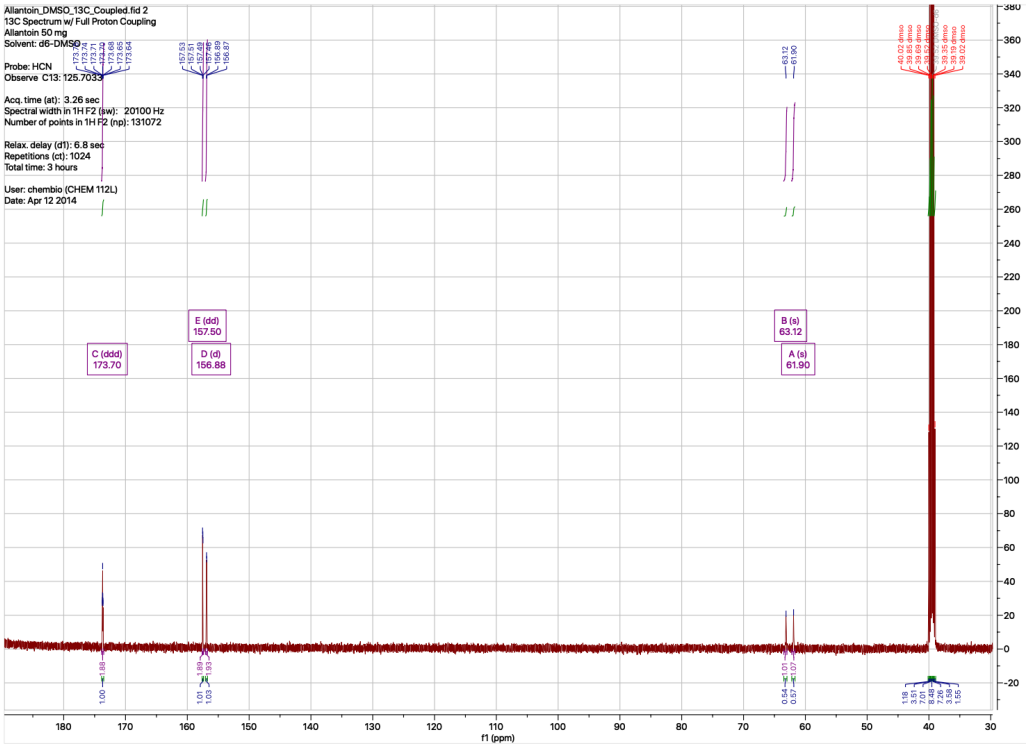

Figure 6: ^{13}C NMR Spectrum of Allantoin in DMSO
This figure highlights the relevant C^{13} NMR peaks for allantoin in DMSO.

Name	Chemical Shift (ppm)	Proton Decoupled Integration	Inverse Gated Integration	Carbon Assignment
A	174.0	0.95	1.03	C5
B	157.6	1.21	1.06	C7
C	157.1	1.04	1.08	C2
D	62.7	1.87	0.99	C4

Figure 7: Assignment of ^{13}C NMR Spectrum for Allantoin in DMSO
This table gives assignments for the ^{13}C NMR of allantoin based on expected chemical shifts and MestReNova estimations. The integrations are also compared between the two different ^{13}C spectrum.

The C7-N6-C5-H5 dihedral angle was calculated from the fully decoupled spectrum and the heteronuclear coupling constant between H5 and neighboring carbons, which was 5.44, according to the Karplus equation to be 154.65°.
The H1-N1-C5-H5 dihedral angle was 104.28° and was calculated from the Karplus equation as well as the smaller coupling constant, 1.39.
The H6-N6-C5-H5 dihedral angle was 156.25° and was calculated from the Karplus equation and the coupling constant of 8.23, this value was provided.

Allantoin DQF-COSY NMR:

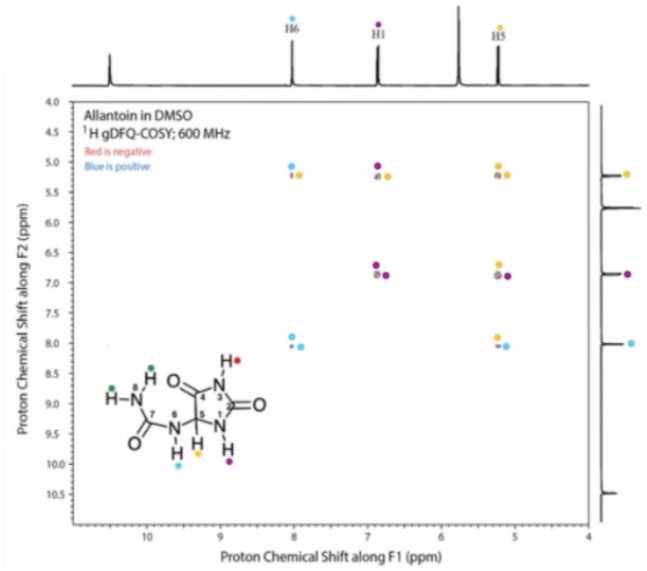

Figure 8: DQF-COSY NMR Spectrum of Allantoin in DMSO
This figure gives the DQF-COSY NMR spectrum for allantoin. The assignments are color coded. This spectrum reveals coupling between hydrogens that are within two or three bonds of each other. This spectrum does not provide information about any heteronuclear correlation or information about ^{13}C NMR.

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Allantoin NOESY NMR:

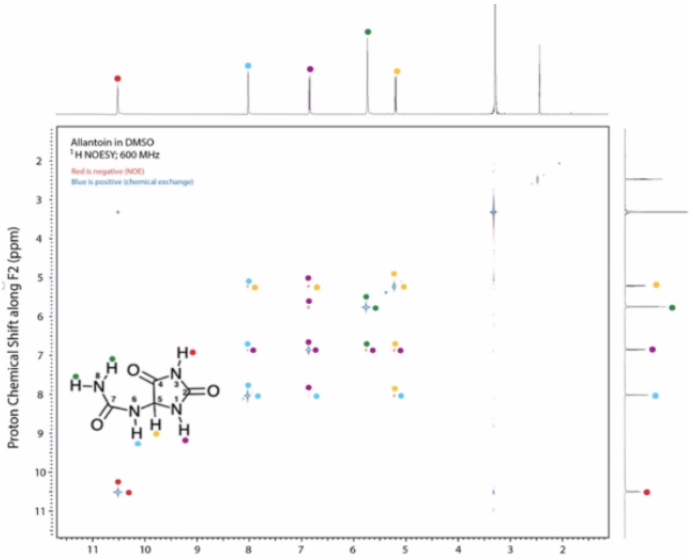

Figure 9: NOESY NMR Spectrum of Allantoin in DMSO

This figure shows the color coded NOESY spectrum for allantoin and gives a full set of NOEs for allantoin. NOESY spectra also provide information on more favorable conformations for molecules because they measure the interactions between atoms based on distance, not bonds.

Allantoin TOCSY NMR:

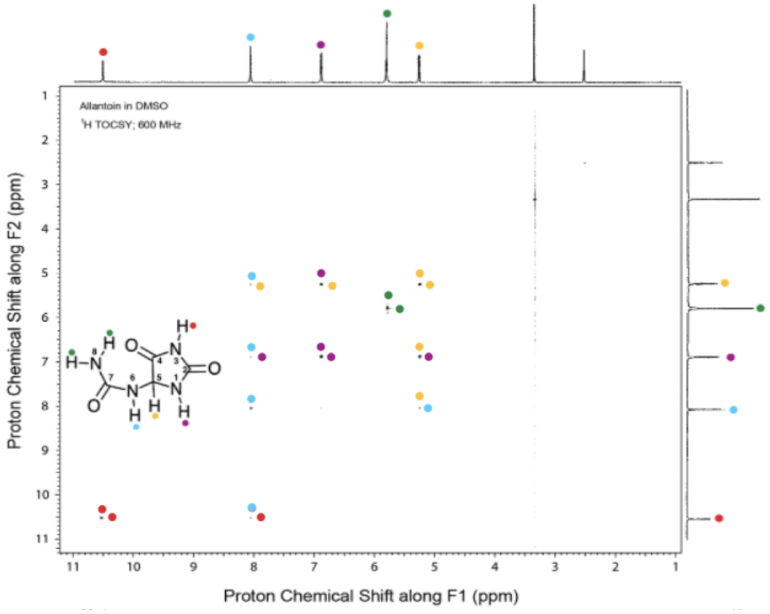

Figure 10: TOCSY Spectrum of Allantoin in DMSO

This figure shows the TOCSY spectra of allantoin and shows the coupled spin system between all of the hydrogen atoms besides the two terminal hydrogens 8a and 8b.

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Allantoin ^{13}C HMBC NMR:

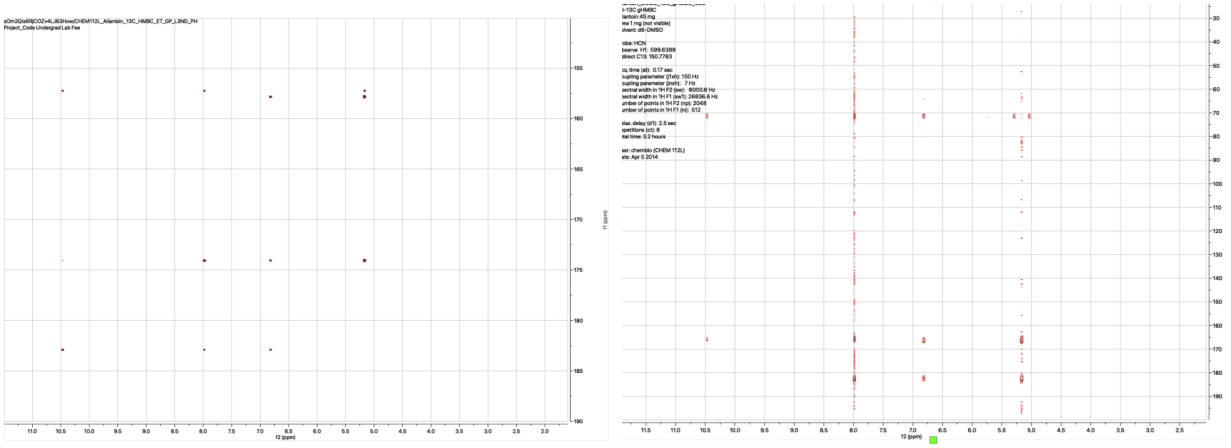

Figure 11: ^{13}C HMBC NMR spectra of Allantoin in DMSO

This figure compares the two ^{13}C HMBC NMR spectra of allantoin in two different instruments. On the left is the ^{13}C HMBC NMR spectrum of allantoin in the 500 MHz Bruker instrument and on the right is the ^{13}C HMBC NMR spectrum of allantoin in the 600 MHz Bruker instrument. Both spectra clearly show that there were mistakes made in the experimental parameter choices. The data being folded over in the 500 MHz instrument is annoying, but this can easily be accounted for. On the other hand, the excessive noise in the 600 MHz spectrum would cause this data on its own to be much more challenging to interpret. For these reasons, I believe that the 500 MHz instrument spectrum is more useful overall.

Allantoin ^{15}N HSQC NMR:

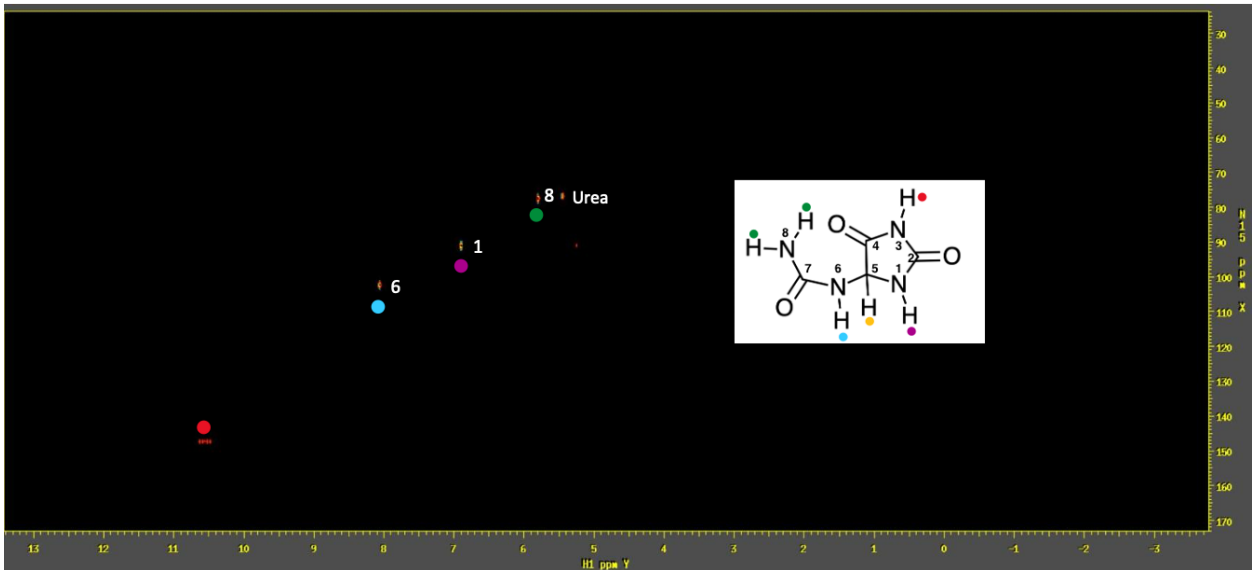

Figure 12: ^{15}N HSQC NMR Spectrum of Allantoin in DMSO

This figure shows the assigned ^{15}N HSQC NMR Spectrum of Allantoin.

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Allantoin ^{15}N HMBC NMR:

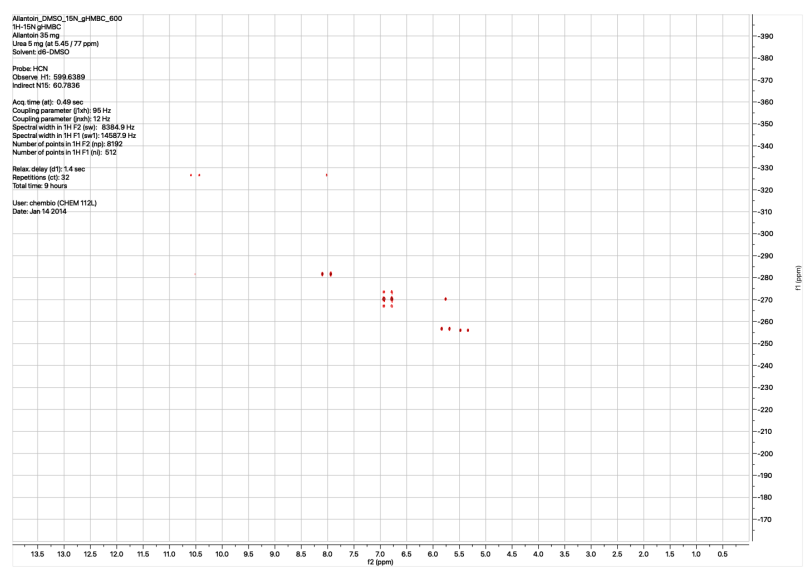

Figure 13: ^{15}N HMBC NMR spectrum of Allantoin in DMSO
This figure shows the ^{15}N HMBC NMR spectrum of Allantoin as well as the correlation between nitrogens and protons that are multiple bonds apart.

Discussion:

Overall the elucidation of the fatty acid methyl ester was fairly straightforward for a couple of reasons, first most of the relevant proton signals were clear and easy to interpret. We have also analyzed similar data earlier in the year. Only the vinylic hydrogens and the many hydrogens of the main chain were challenging to determine the splitting pattern of. The many main chain hydrogens did not really need to be analyzed. The integration value provided all of the information necessary for these atoms. The α-hydrogens were easy to elucidate because they had a simple triplet as would be expected from the two ß-hydrogens. The ß-hydrogens were near two sets of two equivalent hydrogens so the n+1 rule still applied. Similarly, allylic hydrogens have three coupled hydrogens, so the observed four peaks logically follow. Vinylic hydrogens were easily visualized by MestReNova's multiplet analysis as shown below:

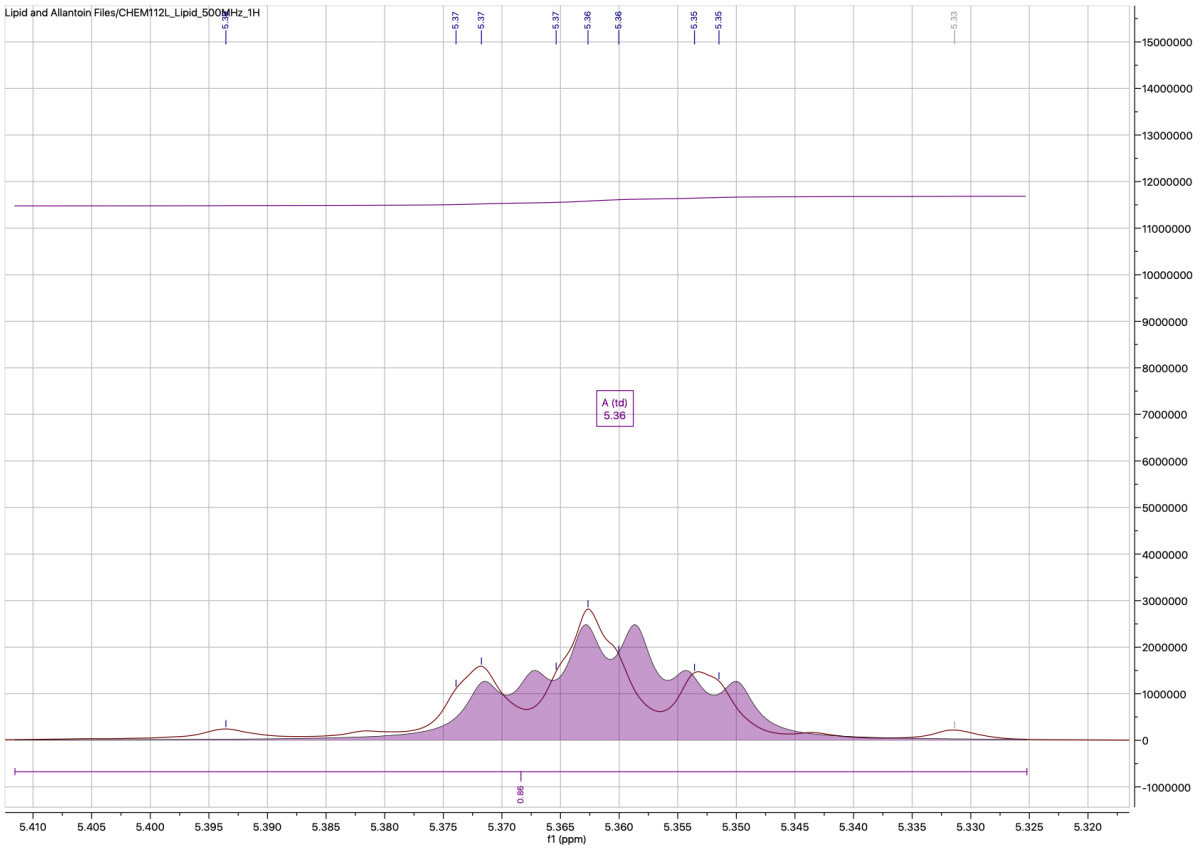

Figure 14: Vinylic ^{1}H NMR Signals in the Fatty Acid Methyl Ester
This figure highlights the unedited spectra of the vinylic carbons with what appears to be a poorly resolved triplet. MestReNova's automatic multiplet analysis instead shows the more logical triplet of doublets.

Allantoin NMR Experiments	Spectral Assignment Elucidation	Conformation Elucidation
Proton NMR (25 ℃ and 50 ℃)	Proton Peak Assignment	Primarily Structural Analysis
Carbon NMR with Proton Coupling	Carbon Peak Assignment	Primarily Structural Analysis
Carbon NMR with Proton Decoupling	Carbon Peak Assignment	Primarily Structural Analysis
Carbon NMR with Inverse Proton Coupling	Carbon Peak Assignment	Primarily Structural Analysis
DQF-COSY NMR	Proton Peak Assignment	Primarily Structural Analysis
NOESY NMR	Proton Peak Assignment	Distances Between Specific Nuclei
TOCSY NMR	Proton Peak Assignment	Coupled Hydrogen Spin Systems
Carbon HMBC NMR	Correlated Proton and Carbon Peak Assignment	Primarily Structural Analysis
Carbon HSQC NMR	Correlated Proton and Carbon Peak Assignment	Primarily Structural Analysis
Nitrogen HMBC NMR	Correlated Nitrogen and Carbon Peak Assignment	Primarily Structural Analysis
Nitrogen HSQC NMR	Correlated Nitrogen and Carbon Peak Assignment	Primarily Structural Analysis

Figure 15: Table of NMR experiment applications
This table highlights the general applications of each of the performed experiments specifically related to peak assignment and conformational elucidation. Note that coupling constants from any experiment can be applied to determining the conformation of a molecule of interest by calculating the dihedral angles between atoms.

Based on the calculated dihedral angles above it is likely that allantoin has a conformer with H1-N1-C5-H5 dihedral angle near 104.28°, a H6-N6-C5-H5 dihedral angle near 156.25°, and a C7-N6-C5-H5 dihedral angle near 154.65°. These values were all obtained from our NMR because the quantum mechanical calculations did not make any sense. Dihedral angles at these values would cause significant unfavorable interactions and would be energetically extremely unlikely to occur. That being said, our NMR spectra were fairly well resolved and the values of the dihedral angles obtained from the simulated coupling constants make sense.

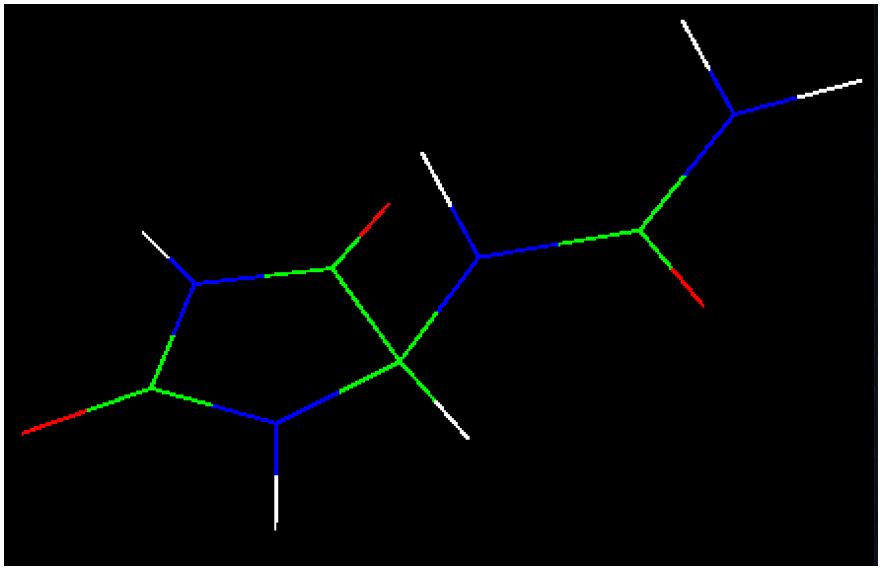

Figure 16 Conformation of Predicted Allantoin Molecule
This figure shows a generated model of the expected orientation of allantoin based on the Karplus equations and spin simulations.

In this experiment we determined a possible conformation of allantoin and determined the spectral assignments for a fatty acid methyl ester. We revisited NMR theory and got a lot of practice interpreting NMR spectra. In particular, we studied many new 2D NMR experiments that provided novel information about the structure and conformations of the molecule of study. I found the most interesting part of this lab to be the use of multiple NMR experiments in order to confirm previous NMR assignments. That is, the more experiments the better. We did not determine anything particularly new about allantoin, but still it was a good educational example because of its relevance to our previous experiments.

Conclusion:

We utilized various 1D and 2D NMR experiments in order to help elucidate the peaks of a fatty acid methyl ester and allantoin. We also used NMR in order to come up with two possible conformations of allantoin. We compared these allantoin conformations to previously quantum mechanically determined allantoin conformers. These experiments contributed to our understanding of the interactions of allantoin and the practical applications of NMR.

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References:

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5. About. http://www.spindynamica.soton.ac.uk/about/ (accessed Jun 2, 2021).