## Background

There are a variety of spectroscopic techniques that will give information about the structure of a molecule. Techniques such as FT-IR and Raman can give information about the functional groups and molecular backbone respectively. However, they cannot give all of the information about the molecule and the environment of the nuclei. Nuclear Magnetic Resonance (NMR) is a powerful technique for providing information about functional groups, molecular backbone AND the chemical environment of the nuclei in the molecule.

The principle of NMR is that the resonance frequency of a nucleus is determined by its gyromagnetic ratio and the strength of the static magnetic field. If this was the only factor determining resonance then nuclei of the same type would have identical frequencies. However, the resonance frequency of a nucleus also depends subtly on its location within a molecule. More precisely it depends on the electron distribution in a molecule and the shielding effect of the surrounding electrons. The shielding is the result of the static magnetic field inducing electron orbital motion. This motion generates a small magnetic field in the opposite direction to the main field. Thus each nucleus experiences a slightly different magnetic field depending on their location in a molecule. This effect is referred to as chemical shift and is the basis for the chemical specificity that is one of the great strengths of NMR spectroscopy.
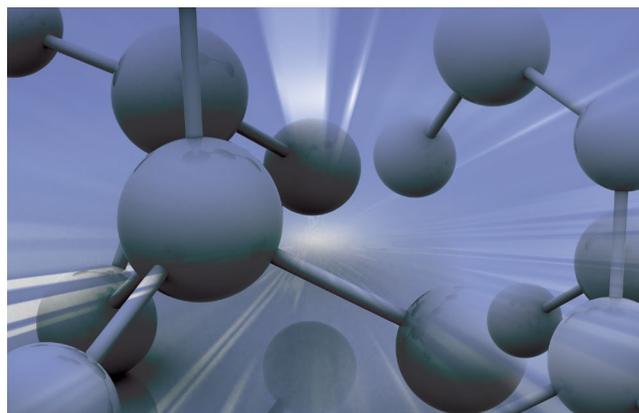
Chemical shift is not the only information contained in a NMR spectrum. The magnetic interaction between neighbouring nuclei mediated through the bonding network is referred to as J-coupling or scalar coupling. This coupling between nuclei results in multiplets in the NMR spectrum. The number of spectral lines and spacing between them in a multiplet provides additional information about the structure of a molecule.

In addition, NMR has the advantage that the amplitude of the NMR signal is directly proportional to the concentrations of the contributing nuclei. Therefore, the ratio of the area under the different peaks corresponds to the number of nuclei per molecule contributing to a resonance. The spectral peak integrals are useful additional information that helps confirm spectral assignments.
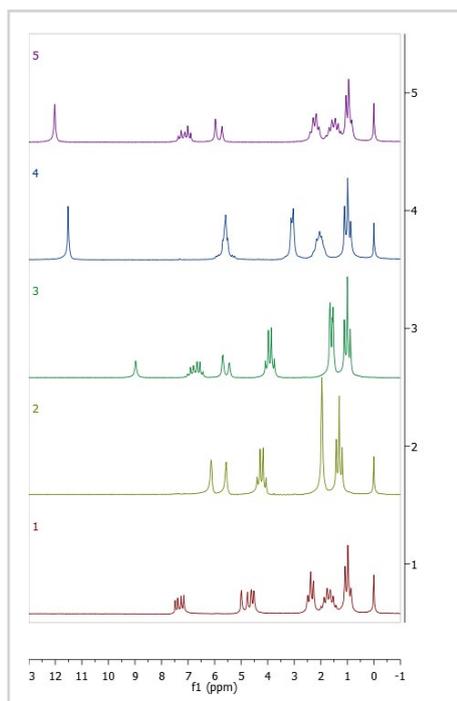
Analysis

To demonstrate the quality of spectra that can be obtained at 1.4 T corresponding to a ^1H resonance frequency of 60 MHz, the ^1H spectrum from 5 small molecules are shown in Figure 1. The molecules all have the same chemical formula $C_6H_{10}O_2$ and contain a double bond and a carboxyl group (-C(=O)O) in the form of an ester (R-C(=O)O-R') or a carboxylic acid (R-C(=O)O-H). 500 mM solutions of each molecule were prepared in $CDCl_3$ and 100 μL were transferred to a 5 mm NMR tube.

Figure 1: Spectra of 5 small molecules with the chemical formula $C_6H_{10}O_2$

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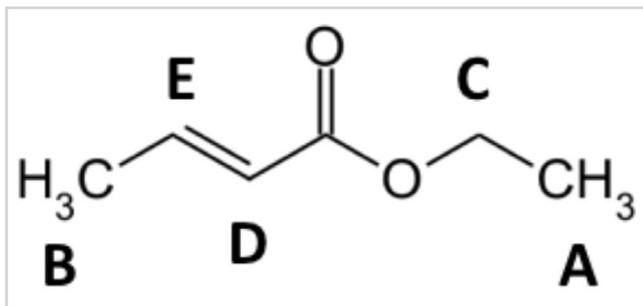## 1. Detailed Interpretation of the Ethyl Crotonate spectrum

Figure 2: chemical structure of ethyl crotonate

The ^{1}H spectrum of ethyl crotonate (figure 2) acquired at 60 MHz is shown in figure 3. The spectrum shows a singlet resonance at 9.23 ppm which can be attributed to the triazine added to the solution to provide a reference signal. There are five other resonances labelled A to E with a range of coupling patterns which can be used for spectral assignment. Resonance A centred at 1.25 ppm is a triplet with a splitting of 7.1 Hz. Resonance B centred on 1.84 is a doublet of doublets with splittings 6.8 Hz and 1.56 Hz. Resonance C centred at 4.16 ppm is a quartet with splitting 7.1 Hz. Resonance D centred 5.8 ppm is a doublet of quartets splitting 15.46 Hz and 1.56 Hz. Resonance E is a doublet of quartets centred at 6.99 ppm with splittings 15.46 Hz and 6.8 Hz. The spectral information is summarised in table 1.

Considering the chemical shifts only and comparing them to typical values for ^{1}H nuclei, resonance A and B are likely to originate from the two methyl groups ($-CH_3$), with resonance C originating from the methylene group ($-CH_2-$) and the source of resonances D and E is the two alkene ^{1}H nuclei.

The splitting pattern of resonances A and B can be used to assign the appropriate methyl groups. The triplet pattern of resonance A and the single splitting imply that the nuclei assigned to resonance A should have two identical neighbouring ^{1}H nuclei, while the doublet of doublets structure in resonance B implies two non-identical neighbouring ^{1}H nuclei with two different splittings. It is now possible to assign resonance A to the methyl ^{1}H nuclei of the ethyl group (CH_3-CH_2-). Further evidence for this assignment

is resonance C which has been assigned to the methylene hydrogens of the ethyl group. The quartet structure implies three identical neighbouring ^{1}H nuclei with the same splitting as resonance A. In fact the triplet, quartet pair of resonances is typical of an ethyl group. Resonance B can be assigned to the second methyl group that is adjacent to the double bond, where the two alkene ^{1}H nuclei are the source of the two different splittings.
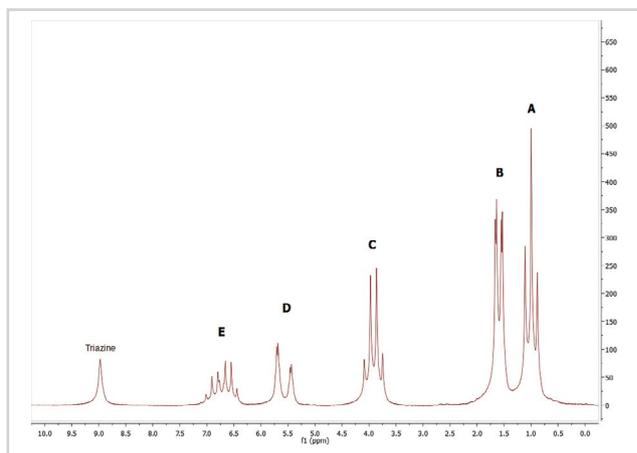

Figure 3: ^{1}H spectrum of 0.5 M ethyl crotonate in CDCl$_3$ acquired at 60 MHz

Splittings across a double bond are typically larger than those across a single bond and the mutual coupling between the two alkene ^{1}H nuclei accounts for the 15.46 Hz splitting. The coupling between two ^{1}H nuclei becomes weaker the greater the number of bonds between them. Resonance E can be assigned to the alkene ^{1}H nuclei closest to the methyl group, accounting for the 6.8 Hz splitting. Resonance D, therefore, can be assigned to the alkene hydrogen nuclei closest to the carboxyl group with the weaker coupling to the methyl group.
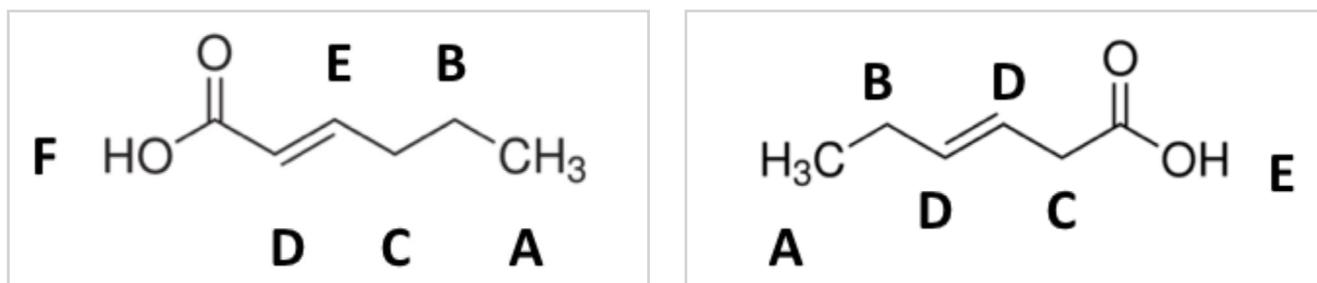
Further evidence for the assignments can be obtained by integrating the area under each of the resonances. Normalising the integral of resonance C to a value of 2 it can be shown that the other resonance correspond to the correct number of nuclei. The integrals for Resonances A and B show inaccuracies due to the overlap in the spectrum.

It is notable in figure 3 that the multiplet patterns of the resonances are not symmetrical and in the case of the ethyl groups ($-CH_2-CH_3$) do not conform to the binomial pattern, 1:3:3:1 and 1:2:1, of peak amplitudes. The asymmetry is particularly obvious in resonance D and resonance E, although it is still noticeable in the other resonances. The source of the asymmetry is strong coupling. At 60 MHz the differences in chemical shift between two neighbouring nuclei is not necessarily much larger than the scalar coupling between them. Under these conditions the weak coupling assumption is no longer valid and coupling patterns associated with weak coupling should not be expected.

Label	δ_H (ppm)	multiplicity	Splitting (Hz)	Integral	assignment
A	1.25	triplet	7.1	3.29 (3)	ethyl $-CH_3$
B	1.84	doublet of doublets	6.8 , 1.56	3.24 (3)	crotonyl $-CH_3$
C	4.16	quartet	7.1	2 (2)	ethyl $-CH_2-$
D	5.80	doublet of doublets	15.5, 1.56	0.96 (1)	=CH C(=O)-
E	6.99	doublet of quartets	15.5, 6.8	0.98 (1)	-CH=
triazine	9.23	singlet	-	-	triazine reference

Table 1: Summary of the spectral information and peak assignments for ethyl crotonate

2. Comparison of Trans-2-hexenoic and trans-3-hexenoic acid spectra

Figure 4: Chemical structures of trans-2-hexenoic acid (left) and trans-3-hexenoic acid (right)

The ^{1}H spectra of trans-2-hexenoic acid (figure 4 left) and trans-3-hexenoic acid (figure 4 right) acquired at 60 MHz are shown in figure 5, with the spectral information summarised in table 2. As with the three esters, by considering the chemical shift, splitting patterns due to scalar coupling and peak integrals the resonances seen in the spectrum of the two carboxylic acids can be assigned to the different ^{1}H nuclei.
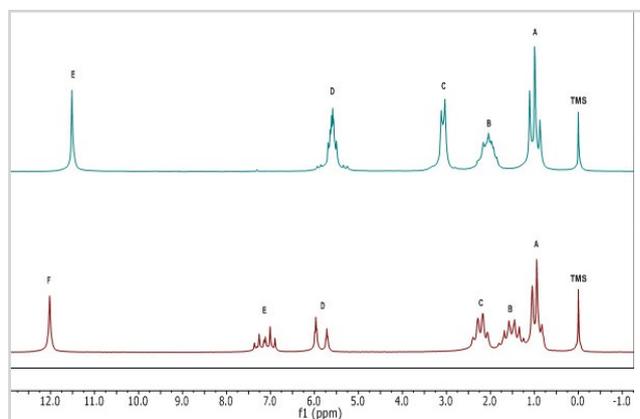
Figure 5: ^{1}H spectrum of 0.5 M trans-2-hexenoic acid (bottom and 0.5 M trans-3-hexenoic acid (top) in $CDCl_3$ acquired at 60 MHz.

There are a number of notable features in the two spectra. Shifting the double bond by one carbon has quite a dramatic effect on the ^{1}H spectrum. The singlet peak close to 12 ppm that is characteristic of a carboxylic acid group, shows a shift of almost 0.5 ppm when the carboxylic acid group is one carbon further away from the double bond. In addition the double bond in the trans-3-hexenoic acid is flanked on either side by a methylene group, the result is that the two alkene hydrogens, –CH= experience very similar chemical environments and as a consequence have very similar chemical shifts. In the spectrum of trans-3-hexenoic acid they are virtually superimposed. In contrast the two alkene hydrogen nuclei in trans-2-hexenoic acid have markedly different chemical shifts and show very different splitting patterns due to their different neighbouring groups.

Label	δ_H (ppm) Trans-2-	δ_H (ppm) Trans-3-	assignments
TMS	0	0	TMS reference
A	0.93	0.98	$-CH_3$
B	1.51	2.01	$-CH_2-$ closest to methyl
C	2.22	3.06	$-CH_2-$ closest to double bond
D	5.82	5.56	=CH-C(=O)-
E	7.12		-CH=
F	11.97	11.5	-COOH

Table 2: Comparison of Chemical Shifts for trans-2- and trans-3-hexenoic acid

Summary

NMR has been shown to be an extremely useful analytical technique for the characterisation of these small molecules. It has been possible to assign the peaks to particular nuclei in the molecule and observe the effect that the environment of the nuclei can have on the chemical shift of the peaks in the NMR spectrum. This is particularly useful in this example where the molecules have the same molecular formula. The differences in the NMR spectra that would be observed with

totally different chemicals with different chain lengths, functional groups and chemical environments would be even greater, although the same principles would apply:

a) The chemical environment of the nucleus will influence the chemical shift of the peaks in the spectrum

b) The peak integrals from the spectrum will indicate the number of nuclei giving rise to that specific peak or peak multiplet.

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