OXFORD INSTRUMENTS

Application Note 7/8

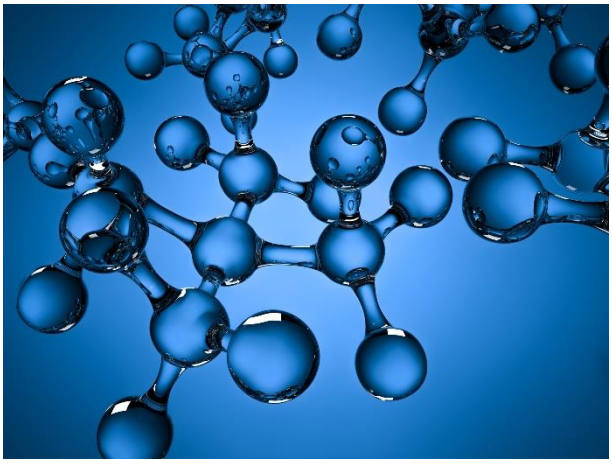
^{1}H-^{1}H COSY & TOCSY two-dimensional NMR spectroscopy

Introduction

One of the great strengths of nuclear magnetic resonance (NMR) spectroscopy, is the wide range of pulse sequences available; and hence different spectra and therefore information which may be obtained.

The basic one-dimensional NMR spectrum comprises a single radio-frequency (RF) pulse followed by acquisition of the NMR signal as a free induction decay (FID) and a Fourier transformation to give the spectrum. By combining multiple RF pulses and varying the delays between them, it is possible to build-up multi-dimensional spectra which provide additional information about the sample.

In this application note we compare the two-dimensional ^{1}H-^{1}H NMR spectra for two structural isomers of $C_6H_{10}O_2$, ethyl crotonate and *trans*-2-hexenoic acid (*Figure 1*); to show the differences between **CO**rrelation **S**pectroscop**Y** (COSY) and **TO**tal **C**orrelation **S**pectroscop**Y** (TOCSY).



^{1}H-^{1}H Two-Dimensional NMR Spectroscopy

An NMR spectrum is produced by performing a discrete Fourier Transformation (dFT) on a series of time domain data points, measured with a particular time interval between each point. It does not matter to the dFT whether the points have been collected in 'real time' or if the series of points have been indirectly constructed. This fact is the basis for two-dimensional (2D) NMR experiments where one dimension is collected in the usual direct manner and a second dimension is constructed in a stepwise manner.

The sequence diagrams associated with two two-dimensional NMR experiments, the COSY and TOCSY, are shown in *Figure* 2. Initially the

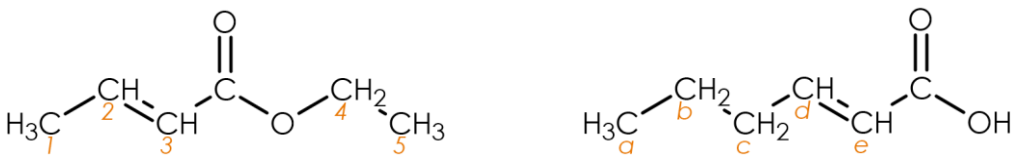


Figure 1 Molecular structures of the $C_6H_{10}O_2$ isomers, Ethyl Crotonate (left) and *trans*-2-Hexenoic Acid (right)

nuclei are excited with a single radio-frequency (RF) pulse to generate a non-equilibrium state. This state is allowed to evolve for a time t_1, before being subjected to further RF manipulation (a single RF pulse for the COSY, a spin-lock for the TOCY). Finally, the NMR signal is recorded (for a time t_2). This process is repeated n times with the value of t_1 being incremented each step, such that the final data is an array of n NMR signals differing only through the duration of the evolution time, t_2. This array then undergoes dFT with respect to the both the times t_1 and t_2 to produce a two-dimensional NMR spectrum.

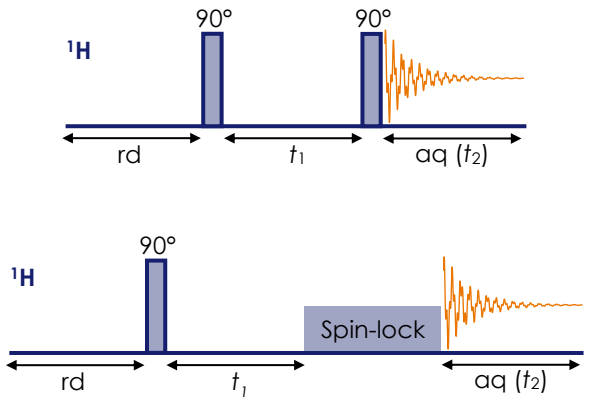


Figure 2 COSY (top) and TOCSY (bottom) pulse sequences

COSY

Homonuclear **CO**rrelation **S**pectroscop**y** (COSY), is the simplest two-dimensional NMR experiment, and correlates the chemical shifts of spins that share a mutual *J*-coupling. Since *J*-coupling is an interaction between nuclei that is mediated through chemical bonds; in general, this coupling gets weaker as nuclei are separated by an increasing numbers of bonds. As a result, the ^{1}H-^{1}H COSY spectrum correlates hydrogen nuclei on adjacent carbons or, in the case of multiply bonded carbons, the next nearest carbons. The method is most commonly used to determine the underlying structure of the carbon back bone in an organic molecule.

All signals that appear in the one-dimensional spectrum will show a peak along the diagonal in the COSY spectrum. The cross-peaks (off diagonal peaks) show which hydrogens share a *J*-coupling through the correlation between the two chemical shifts.

For ethyl crotonate (*Figure 3*), the two ethyl signals *4*-CH_2 and *5*-CH_3 are show to couple together, through a three-bond (H–C–C–H) coupling. While the three signals from the crotonate group, *1*-CH_3, *2*-CH & *3*-CH, couple

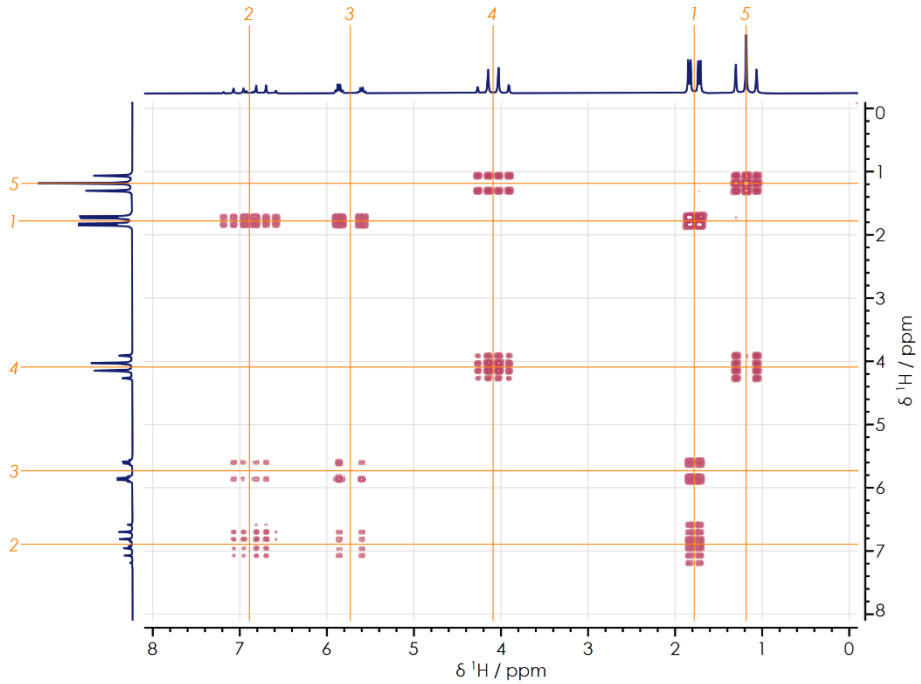


Figure 3 ^{1}H-^{1}H COSY spectrum of Ethyl Crotonate

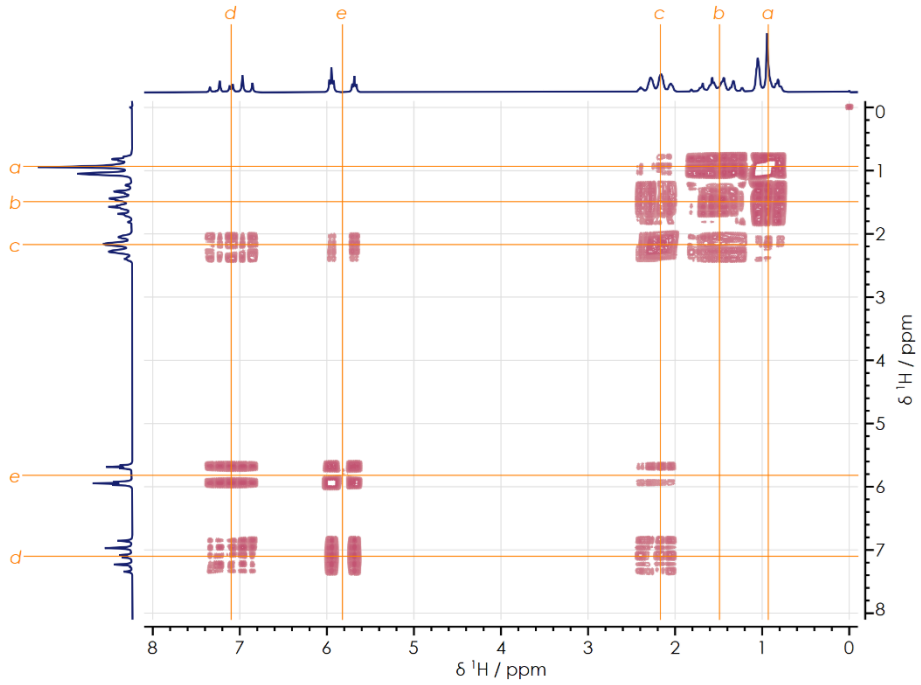


Figure 4 ^{1}H-^{1}H COSY spectrum of *trans*-2-Hexenoic Acid

through three- (H–C–C–H, H–C=C–H) or four- (H–C–C=C–H) bond interactions.[1]

While for *trans*-2-hexenoic acid (*Figure 4*), the five signals from the hydrocarbon backbone, *a* to *e*, which all show peak splitting arising from *J*-coupling to nearby hydrogens.[2] The COSY spectrum reveals the three-bond coupling between *a*-CH_3 & *b*-CH_2, *b*-CH_2 & *c*-CH_2, *c*-CH_2 & *d*-CH and *d*-CH & *e*-CH. It also shows the four-bond coupling between *c*-CH_2 & *e*-CH.[3]

TOCSY

TOtal **C**orrelation **S**pectroscop**Y** (TOCSY), is a homonuclear two-dimensional experiment similar to COSY, in which the *J*-coupling between two hydrogen nuclei manifests as a cross peak in the spectrum. Unlike COSY, however, the detection of the coupled spins is not limited to nearest neighbours. The TOCSY experiment exploits isotropic mixing which occurs during spin-locking to produce cross peaks between all hydrogen nuclei that form part of an unbroken chain of coupled spins.

This can be illustrated by considering the TOCSY spectra for ethyl crotonate and *trans*-2-hexenoic acid, and comparing them with the corresponding COSY spectra discussed in the previous section.

If we compare the COSY (*Figure 3*) and TOCSY (*Figure 5*) spectra of ethyl crotonate; we can see that the ethyl crotonate comprises two separate chains of spins, the crotonyl group (*1*-CH_3, *2*-CH & *3*-CH), and the ethyl group (*4*-CH_2 and *5*-CH_3). The lack of cross peaks between the two sets of spins confirms the presence of nuclei which effectively breaks the *J*-coupling chain. In this case those of the ester {–C(O)O–} linkage.

In contrast, the TOCSY spectrum of *trans*-2-hexenoic acid (*Figure 6*) shows coupling between all pairs of hydrogen nuclei, as the hydrocarbon backbone (*a* to *e*) comprises a single unbroken chain of coupled spins.

[1] Four-bond couplings are usually fairly week and not observed; in this case the double bond between *2*-CH & *3*-CH, ensures the coupling between *1*-CH_3 & *3*-CH is large enough to observe ($^{4}J_{HH}$ = 1.6 Hz).

[2] There's a sixth signal in the full *trans*-2-hexenoic acid spectrum from the carboxylic acid group, this does not interact with any other protons, and occurs at a chemical shift (δ_H) of around +12 ppm.

[3] As for the ethyl crotonate, this is due to the case the double bond between *d*-CH & *e*-CH.

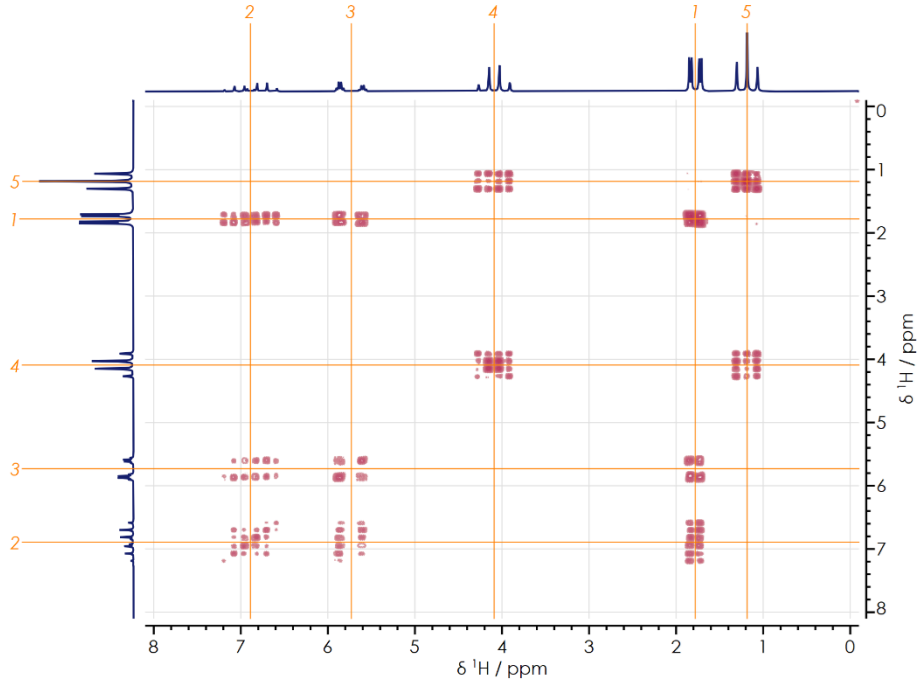


Figure 5 ^{1}H-^{1}H TOCSY spectrum of Ethyl Crotonate

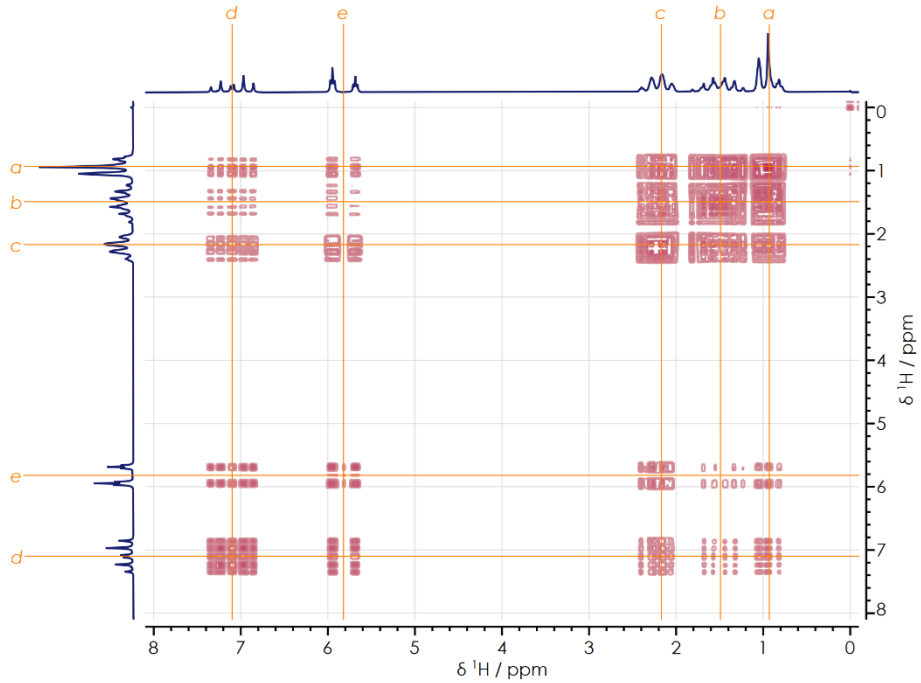


Figure 6 ^{1}H-^{1}H COSY spectrum of *trans*-2-Hexenoic Acid

Summary

COSY and TOCSY are only two of the commonly used one- and two-dimensional NMR experiments used for structural elucidation of unknown chemicals (see *X-Pulse Application Notes 1, 14, & 21*, for examples of other one- and two-dimensional NMR experiments).

The **Oxford Instruments X-Pulse Broadband Benchtop NMR Spectrometer** comes with three-axis pulse-field gradients *as standard*, permitting the more efficient and effective gradient-selective versions of two-dimensional correlation experiments to be used. Combined with the optional twenty five position autosampler, efficiency and throughput can be maximised.

If you have any questions about this application note, please contact our experts:
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