School of Chemistry, Food and Pharmacy

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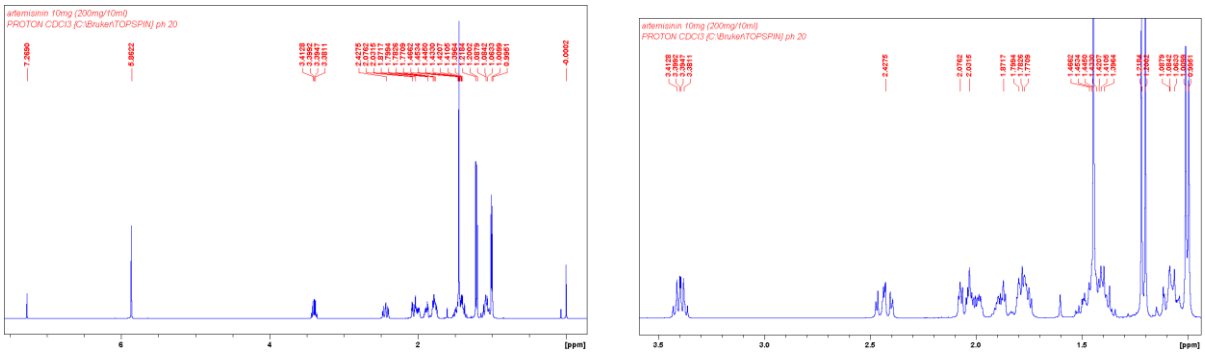
Interpretation of Experiments from ICONNMR

By using some or all of the seven experiments (^{1}H, ^{13}C, DEPT, HSQC, HMBC, COSY and NOESY) which are routinely available in ICON NMR in conjunction with one other, it should generally be possible to solve the structure of an organic compound in more than 90% of cases, provided that sufficient sample is available (see Sample Requirements for Experiments in ICONNMR).

Artemisinin ($C_{15}H_{22}O_5$) as an Example of the Interpretation of Spectra from the Open Access NMR Instruments

Artemsinin is a medium-sized organic compound (M_r=282) which is used as an example of the general strategy for solving the structure of an organic compound by use of the ICON NMR Experiments that are available by default with the Open Access NMR Instruments. The structure and complete NMR assignments for artemisinin are shown below (^{1}H chemical shifts are between 1-10 ppm; ^{13}C chemical shifts are between 10-200 ppm).

Interpretation of the 1H NMR Spectrum of Artemisinin

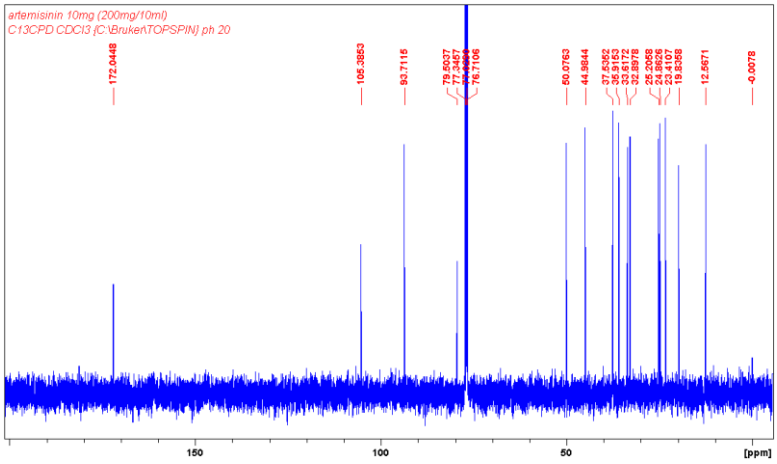


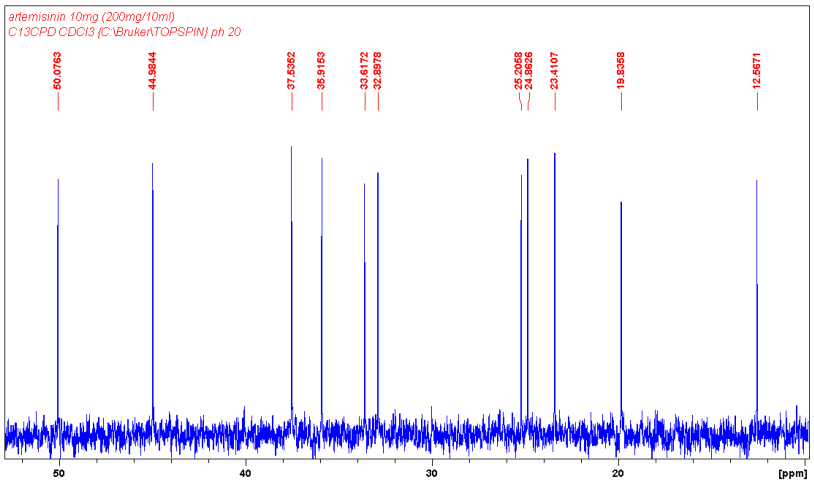
Try to identify as many individual resonances as possible from the ^{1}H NMR spectrum, noting the number of protons associated with each resonance (the integral), the splitting pattern, and the associated coupling constants. It may not always be possible to identify every single ^{1}H NMR resonance. This is the case for much of the proton spectrum of artemisinin between 1-2ppm. In this example, accidental overlap of chemical shifts systems has made it impossible to clearly see multiplet structures for most of the methylene and methine protons contained in the rings of artemisinin. Do not be too concerned by this, as it will become possible to identify all these resonances at a later stage, following analysis of the 2D-NMR spectra. The "first pass" analysis of the 1D- ^{1}H NMR spectrum of artemisinin is shown in the Table below:

5.86	s		H-5
3.40	dq	J=7,7 Hz	H-11
2.43	ddd	J=15,13,4 Hz	H-3
2.06	ddd	J=15,4,4 Hz	H-3
1.45 [3H]	s		H-15
1.21 [3H]	d	J=7 Hz	H-13
1.00 [3H]	d	J=6 Hz	H-14

Interpretation of the 13C NMR Spectrum of Artemisinin

Interpretation of the ^{13}C NMR spectrum simply requires the listing of all fifteen ^{13}C peaks which appear in the ^{13}C NMR spectrum of artemisinin, as shown below:

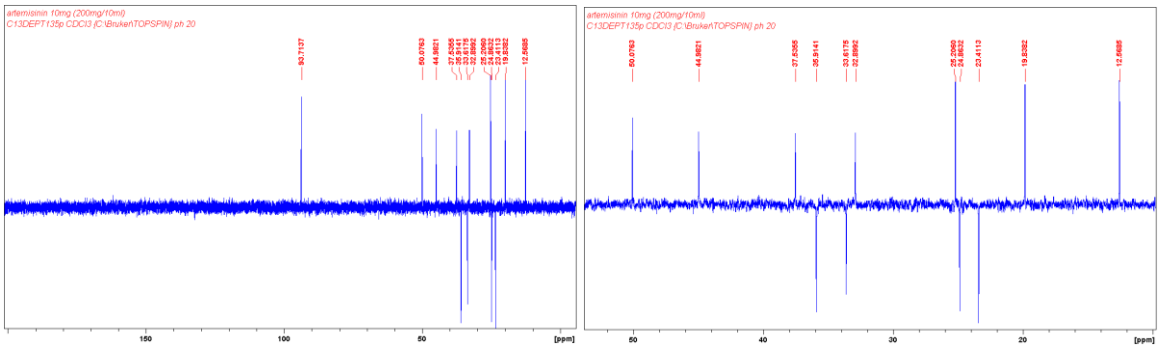




172.1 (C-12)
105.4 (C-4)
93.7 (C-5)
79.5 (C-6)
50.1 (C-1)
45.0 (C-7)
37.5 (C-10)
35.9 (C-3)
33.6 (C-9)
32.9 (C-11)
25.2 (C-15)
24.9 (C-2)
23.4 (C-8)
19.8 (C-14)
12.6 (C-13)

Interpretation of the DEPT-135 Spectrum of Artemisinin

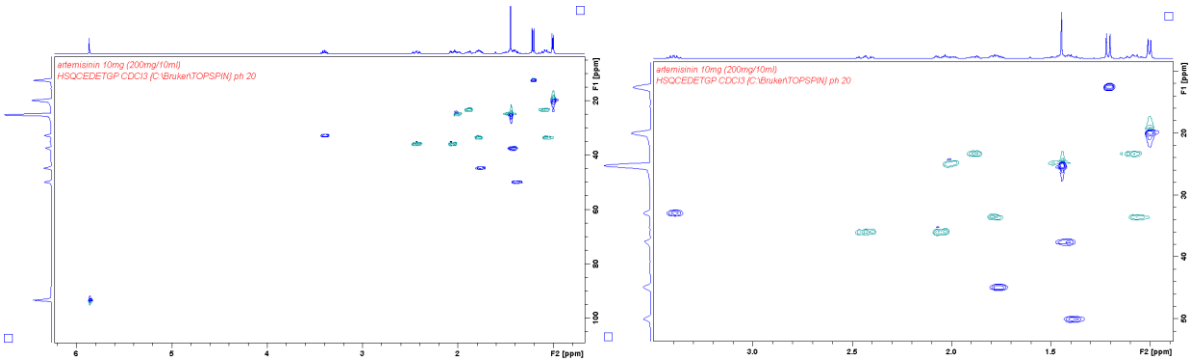
A DEPT-135 spectrum is an "edited" version of a ^{13}C NMR spectrum, in which methylene groups (CH_2) appear negative; methine (CH) and methyl (CH_3) groups both appear positive; and quaternary carbons do not appear at all. Using this information it is possible to assign the number of directly attached protons for the fifteen carbons in artemisinin, as shown in the Table below:



172.1	C (C-12)
105.4	C (C-4)
93.7	CH/CH_3 (C-5)
79.5	C (C-6)
50.1	CH/CH_3 (C-1)
45.0	CH/CH_3 (C-7)
37.5	CH/CH_3 (C-10)
35.9	CH_2 (C-3)
33.6	CH_2 (C-9)
32.9	CH (C-11)
25.2	CH_3/CH (C-15)
24.9	CH_2 (C-2)
23.4	CH_2 (C-8)
19.8	CH_3/CH (C-14)
12.6	CH_3/CH (C-13)

Interpretation of the Edited-HSQC Spectrum of Artemisinin

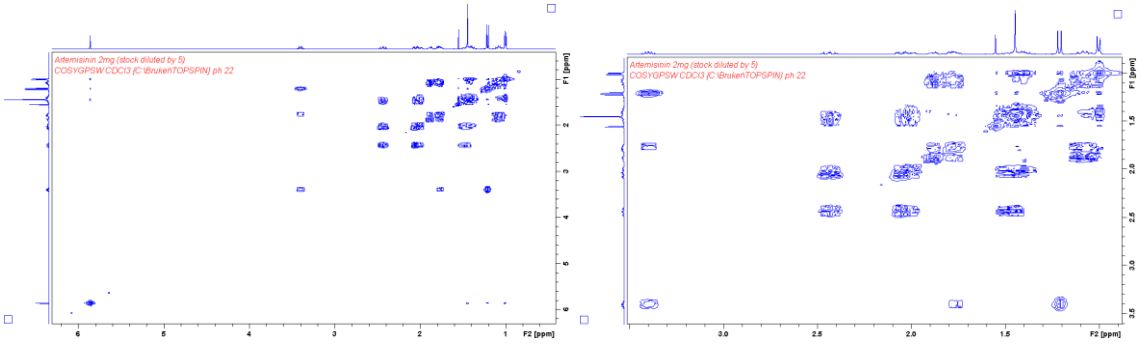
Using the edited-HSQC experiment, it is possible to identify which protons are directly attached to each of the carbons in artemisinin. Because artemisinin contains three quaternary carbons (see the results of ^{13}C NMR and DEPT-135), connections are seen to only 12 of these carbons in the edited-HSQC experiment. Green (negative) peaks correspond to CH_2 groups, and in this case, all methylene carbons are "connected" to two chemically different protons. Blue (positive) peaks correspond to both CH and CH_3 groups, although in this case (unlike the DEPT-135 spectrum) it is possible to distinguish which is which, because the connections to methyl carbons are more intense, and the associated hydrogen resonance have already been identified as having an integral corresponding to [3H]. The 12 "pieces" of the artemisinin molecule which are identified in the edited-HSQC experiment are shown below:



Note that, in addition, it is now possible to unambiguously identify the resonant position of **all** 22 protons in artemisinin. This is a consequence of the increased dispersion which is afforded by the second dimension of the edited-HSQC experiment. The Table below expands the previous analysis of ^{1}H chemical shift which was obtained from the preliminary analysis of the 1D ^{1}H-NMR experiment:

5.86	s		H-5
3.40	dq	J=7,7 Hz	H-11
2.43	ddd	J=15,13,4 Hz	H-3
2.06	ddd	J=15,4,4 Hz	H-3
2.00			H-2
1.88			H-8
1.78			H-9
1.76			H-7
1.47			H-2
1.45 [3H]	s		H-15
1.42			H-10
1.39			H-1
1.21 [3H]	d	J=7 Hz	H-13
1.08			H-8
1.08			H-9
1.00 [3H]	d	J=6 Hz	H-14

Interpretation of the COSY Spectrum of Artemisinin

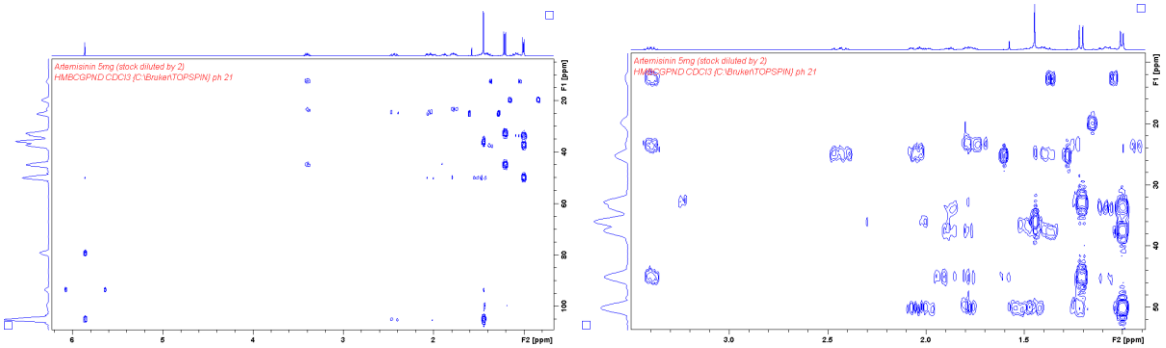


The 2D- COSY spectrum shows two kinds of peaks: those on the diagonal, which have the same chemical shift in both dimensions (and therefore yield no more information than a 1D- ^{1}H NMR spectrum), and the off-diagonal peaks which identify protons coupled to other protons - often via three bonds. A list of the most obvious three-bond correlations which can be deduced from the COSY spectrum of artemsisinin is given in the Table below.

5.86 (H-5)	
3.40 (H-11)	1.76 (H-7), 1.21 (H-13)
2.43 (H-3)	2.06 (H-3), 2.00 (H-2), 1.47 (H-2)
2.06 (H-3)	2.43 (H-3), 2.00 (H-2), 1.47 (H-2)
1.45 [3H] (H-15)	
1.21 [3H] (H-13)	3.40 (H-11)
1.00 [3H] (H-14)	1.42 (H-10)

Although only those most obvious resonances which were previously identified from the 1D ^{1}H-NMR spectrum of artemisinin, have been included in this Table, it is possible to expand this correlation table to include all 22 resonances which wre identified in the edited-HSQC table (this is left as an exercise for the interested student!..). Use of the COSY experiment allows some of the pieces identified from HSQC to be joined up into larger fragments of the molecule, rather like the process for assembling a jigsaw.

Interpretation of the HMBC spectrum of artemisinin



The HMBC spectrum identifies ^{13}C and ^{1}H resonances which are connected by either 2- or 3-bonds. The HMBC spectrum therefore enables us to join together the individual pieces of the molecule which have been determined from the edited-HSQC spectrum. A summary of the correlation information available from this HMBC spectrum is given in the Table below:

172.1 (C-12)	5.86 (H-5), 3.40 (H-11), 1.76 (H-7), 1.21 (H-13)
105.4 (C-4)	5.86 (H-5), 2.43 (H-3), 2.06 (H-3), 1.45 (H-15)
93.7 (C-5)	1.76 (H-7)
79.5 (C-6)	5.86 (H-5), 2.00 (H-2), 1.88 (H-8) , 1.76 (H-7)
50.1 (C-1)	5.86 (H-5), 2.00 (H-2), 1.76 (H-7), 1.47 (H-2), 1.00 (H-14)
45.0 (C-7)	3.40 (H-11), 1.88 (H-8), 1.78 (H-9), 1.21 (H-13)
37.5 (C-10)	1.78 (H-9), 1.39 (H-1), 1.00 (H-14)
35.9 (C-3)	2.00 (H-2), 1.45 (H-15)
33.6 (C-9)	1.88 (H-8), 1.00 (H-14)
32.9 (C-11)	1.21 (H-13)
25.2 (C-15)	2.43 (H-3), 2.06 (H-3)
24.9 (C-2)	2.06 (H-3), 1.39 (H-1)
23.4 (C-8)	3.40 (H-11), 1.76 (H-7)
19.8 (C-14)	
12.6 (C-13)	3.40 (H-11)

The chief complication in the interpretation of the HMBC experiment is that it is not possible to know *a priori* whether a correlation corresponds to a two- or a three-bond connection. This issue can be resolved to some extent by using this experiment in conjunction with COSY. Thus, three carbons – 50.1 (C-1), 37.5 (C-10) and 33.6 (C-9) – all show a correlation to the methyl proton doublet at 1.00 ppm (H-14), and must therefore correspond C-1, C-9 and C-10, if only two- and three-bond couplings are allowed. However, we already know from the COSY spectrum that the H-14 methyl doublet at 1.00 ppm is coupled to a methine proton at 1.42 for H-10. From the HSQC spectrum, we also know that the H-10 proton at 1.42ppm is associated with the carbon at 37.5. Therefore 37.5 must be the C-10 carbon involved in the 2-bond carbon-proton connection, and 50.1 and 33.6 correspond either to C-1 or C-9 via three-bond couplings.

The greatest strength of the HMBC experiment is its ability to complete the" jigsaw" by "connecting up" the quaternary carbons, which cannot be assigned from proton-proton COSY spectrum. Thus, the quaternary carbon at 105.4 (C-4) shows a connection to the methyl singlet at 1.45 (H-15) from

which we can confidently assign this carbon as C-4 (via a 2-bond coupling). A second quaternary carbonyl at 172.1 (C-12) correlates both to a methyl group at 1.21 (H-13) and to a methine proton at 5.86 (H-5), which thereby identifies this carbonyl as C-12, involved in 3-bond couplings to both hydrogens.