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Case Study:
Structure Verification of Quinine Using 1D and 2D NMR Methods

Introduction

Quinine ($C_{20}H_{24}N_2O_2$, MW 324.42 g mol^{-1}, Figure 1) is a drug used to treat a variety of conditions, most notably malaria. It is listed as one of the WHO's (World Health Organization's) "Essential Medicines".[1]

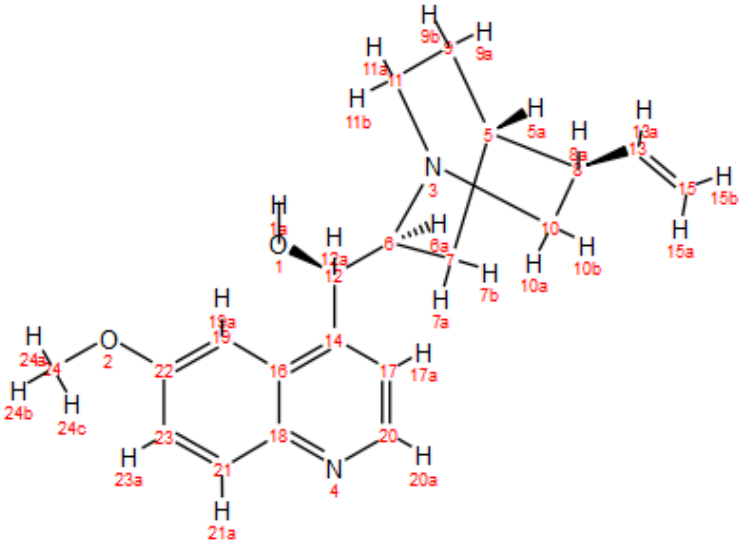

Figure 1. Structure of quinine

In this Case Study, it is shown how a combination of 1D and 2D NMR techniques at 80 MHz can be used to fully and unambiguously assign the 1H and 13C peaks of quinine.

Sample and System

130 mg quinine was dissolved in 1 mL DMSO-d6 to give a quinine concentration of 400 mM. ^{1}H and ^{13}C spectra were collected on a Spinsolve 80 spectrometer with a ^{1}H frequency of 80.27 MHz and a ^{13}C frequency of 20.19 MHz.

NMR Experiments

Table 1 below lists the experiments and main parameters used to assign the peaks of quinine.

Protocol	Scans	NP in t_2	NP in t_1	Aquisition time (s)	Repetition Time (s)	Total time (min.)
Proton +	8	16,384	...	3.2	10	1.3
Carbon +	512	8,192	...	1.6	3	25.6
HSQC-ME	4	1,024	128	0.5	1	17
DQF-COSY	8	2,048	128	1.0	2	75.4
HMBC	32	2,048	128	1.0	2	273
TOCSY	4	2,048	128	1.0	2	35.2
ROESY	4	4,096	128	0.8	2	35.2

Table 1. NMR experiments and parameters used to assign the peaks of quinine

^{1}H NMR Spectrum

Figure 2 shows the ^{1}H NMR spectrum of quinine.

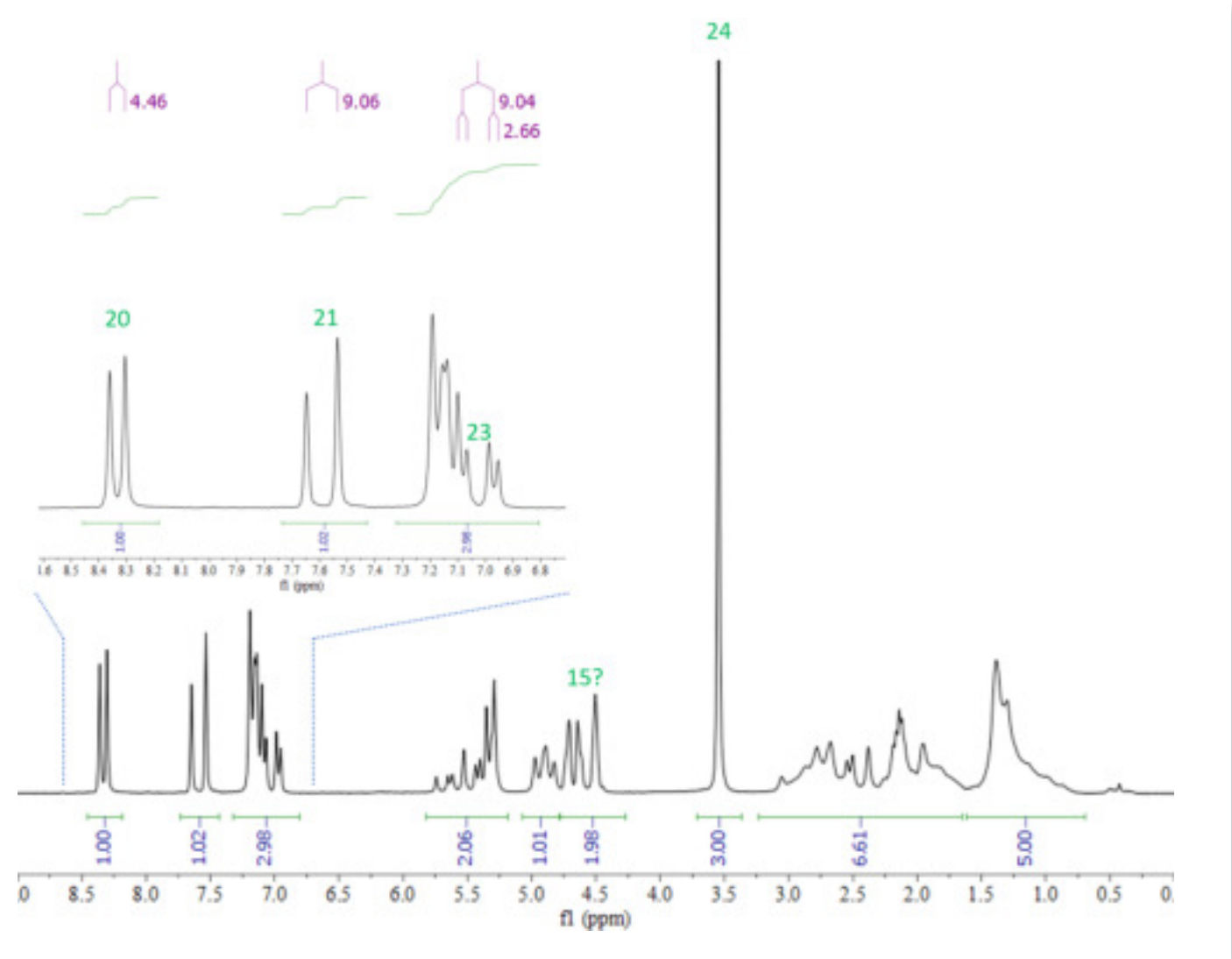

Figure 2. ^{1}H NMR spectrum of quinine

Several resonances in the ^{1}H spectrum can be positively or tentatively assigned based on their chemical shifts, J-splitting patterns and coupling constants, and integrals.

Atom	1H (ppm)	Peak Splitting Pattern	j (Hz)
24	3.55	...	...
20	8.33	d	4.5
21	7.59	d	9.1
23	7.03	dd	9.1, 2.7
15 (tent.)	4.6	m	...

2D methods will be used to confirm assignments and assign the remaining resonances.

^{13}C NMR Spectrum

Figure 3 shows the ^{13}C spectrum of quinine.

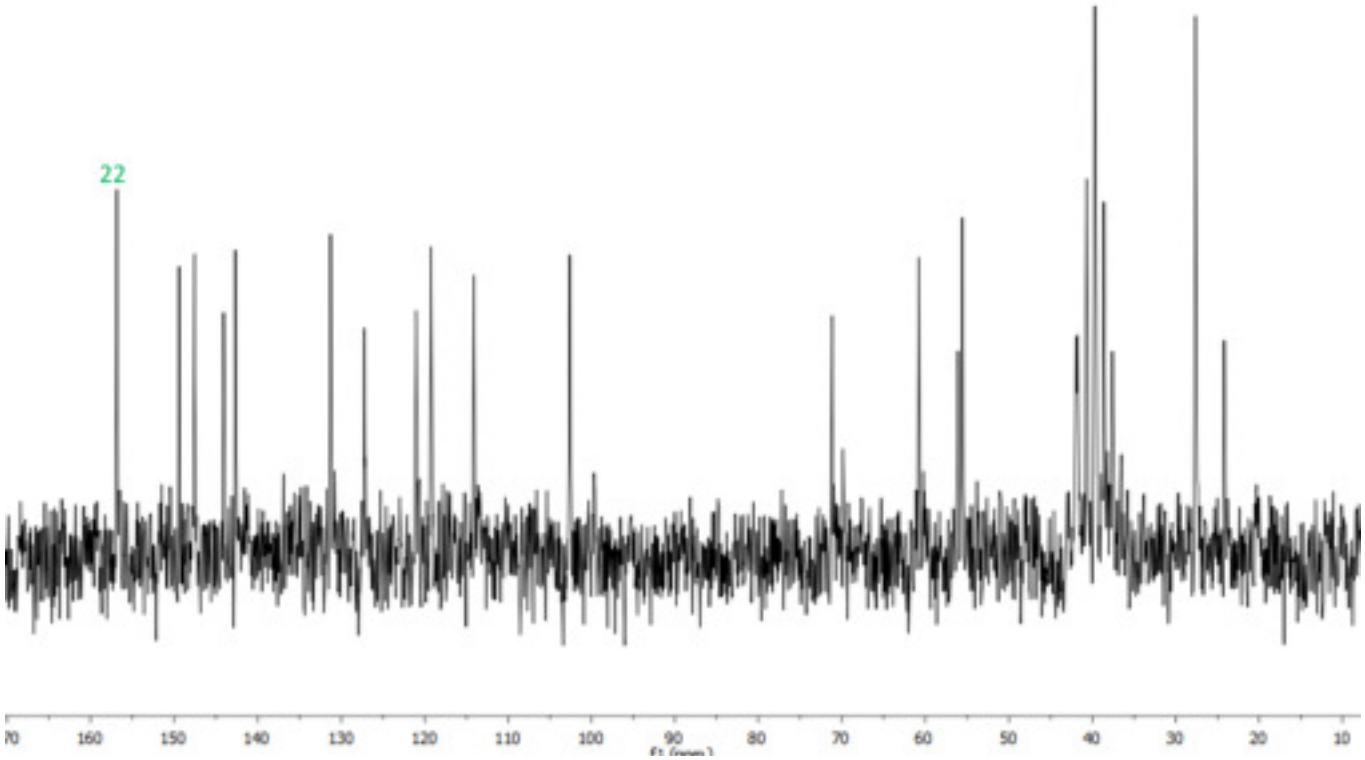

Figure 3. ^{13}C NMR spectrum of quinine

In the ^{13}C spectrum, 17 of the expected 20 peaks of quinine can be clearly observed. However, the intense peak at 27.6 ppm is almost certainly due to two carbon peaks that have the same chemical shift. In addition, some resonances may be obscured beneath the septet signal centered 39.5 ppm from the DMSO-d6 solvent. The downfield signal at δ = 157.3 ppm can be assigned to C22 based on its chemical shift.

^{1}H-^{13}C Multiplicity-Edited HSQC (HSQC-ME) Spectrum

Figure 4 shows the ^{1}H-^{13}C HSQC spectrum of quinine.

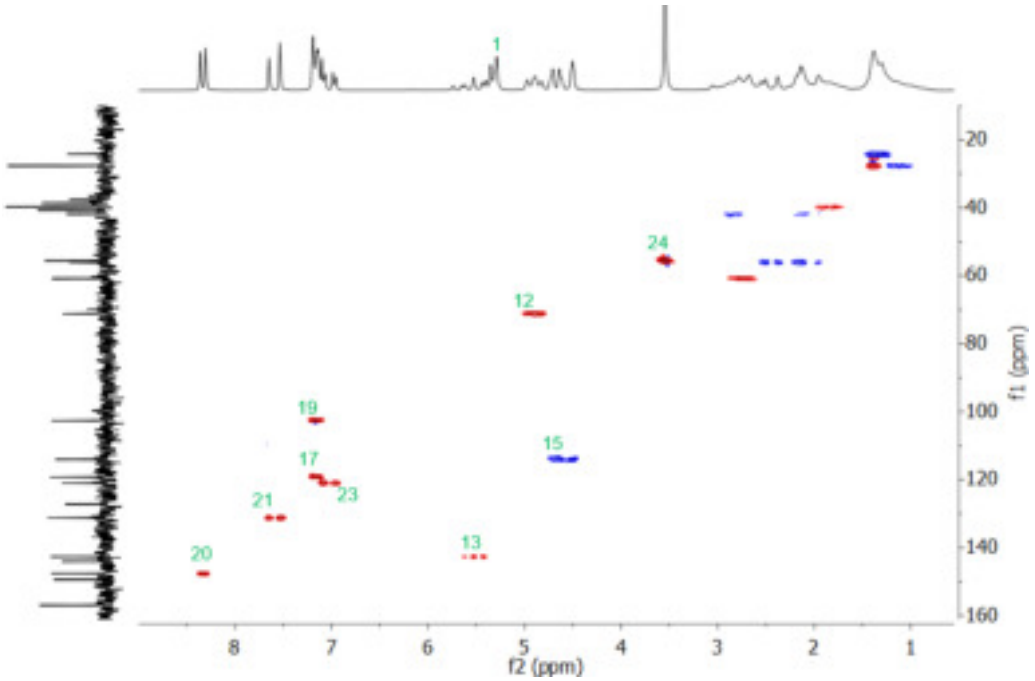

Figure 4. ^{1}H-^{13}C multiplicity-edited HSQC spectrum of quinine

In the HSQC-ME spectrum (Figure 4), the red peaks indicate CH_3 or CH carbons and the blue peaks indicate CH_2 carbons. The spectrum shows that there are 5 CH_2 carbons, consistent with the structure of quinine. The associated ^{13}C chemical shifts of peaks assigned in the ^{1}H spectrum and additional assignments can be made.

Atom	1H (ppm)	^{13}C (ppm)
24	3.55	55.6
20	8.33	147.6
21	7.59	131.3
23	7.03	121.0
15 (tent.)	4.6	114.1
22	...	157.3

Atom	1H (ppm)	^{13}C (ppm)
17 (tent.)	7.16	119.2
19 (tent.)	7.16	102.6
13	5.6	142.7
1	5.3	...
12 (tent.)	4.9	71.5

DQF-COSY Spectrum

A region of the Double-Quantum Filtered (DQF)-COSY spectrum of quinine is shown in Figure 5.

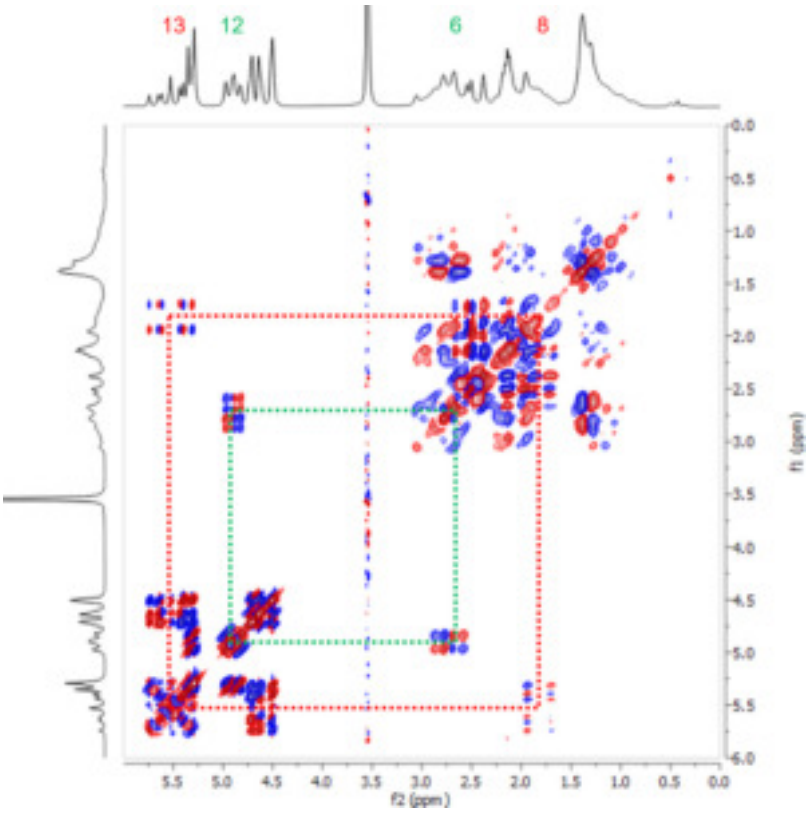

Figure 5. Region of the DQF-COSY spectrum of quinine

DQF-COSY is a phase-sensitive, high-resolution variant of COSY. In addition, the double-quantum filter removes singlet signals that are often intense and can obscure useful cross-peak information. Several additional assignments can be made, and tentative ones confirmed, from the DQF-COSY spectrum. Cross-referencing with the HSQC spectrum provides ^{13}C shifts for those assigned atoms.

Atom	1H (ppm)	^{13}C (ppm)
24	3.55	55.6
20	8.33	147.6
21	7.59	131.3
23	7.03	121.0
15	4.6	114.1
22	...	157.3
6	2.73	60.8

Atom	1H (ppm)	^{13}C (ppm)
17	7.16	119.2
19	7.16	102.6
13	5.6	142.7
1	5.3	...
12	4.9	71.5
8	1.84	39.7

^{1}H-^{13}C HMBC Spectrum

The ^{1}H-^{13}C HMBC spectrum of quinine is shown in Figure 6.

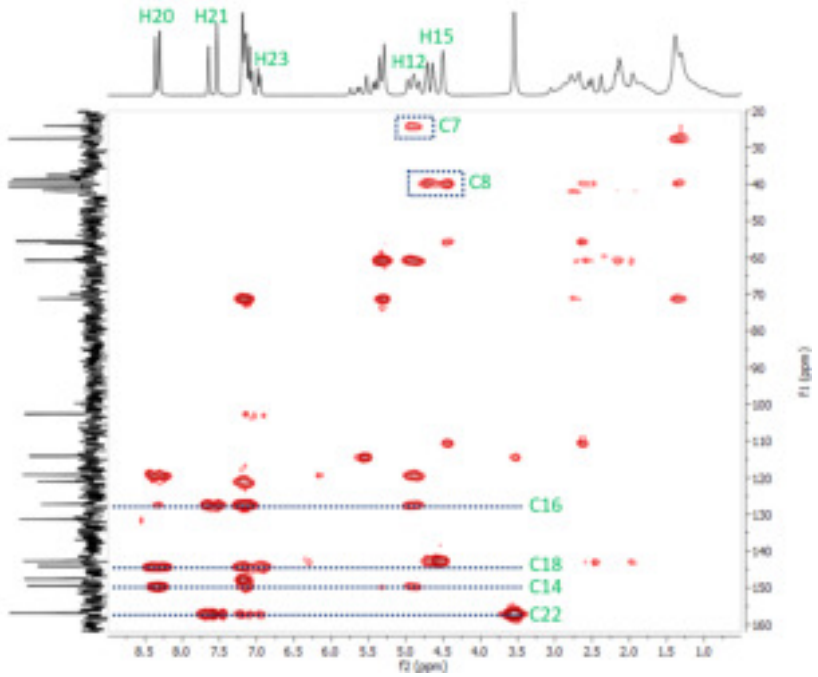

Figure 6. ^{1}H-^{13}C HMBC spectrum of quinine. The experiment was optimized for 10 Hz long-range ^{1}H-^{13}C couplings

HMBC (Heteronuclear Multiple-Bond Correlation) correlates ^{1}H and ^{13}C over more than one chemical bond (typically two or three, but sometimes more). It is useful for establishing connectivities between different fragments of a molecule. It is also the best method for identifying and assigning quaternary carbons. The large number of cross-peaks in typical HMBC provides a wealth of structural information. Many additional and confirmatory assignments of quinine can be made using HMBC, some of which are indicated in Figure 6.

Atom	1H (ppm)	^{13}C (ppm)
24	3.55	55.6
20	8.33	147.6
21	7.59	131.3
23	7.03	121.0
15	4.6	114.1
6	2.73	60.8
18	...	144
22	...	157.3
7	1.33	24.2

Atom	1H (ppm)	^{13}C (ppm)
17	7.16	119.2
19	7.16	102.6
13	5.6	142.7
1	5.3	...
12	4.9	71.5
8	1.84	39.7
14	...	149.4
16	...	127.2
5	1.33	27.6

TOCSY Spectrum

Figure 7 shows a region of the TOCSY spectrum of quinine.

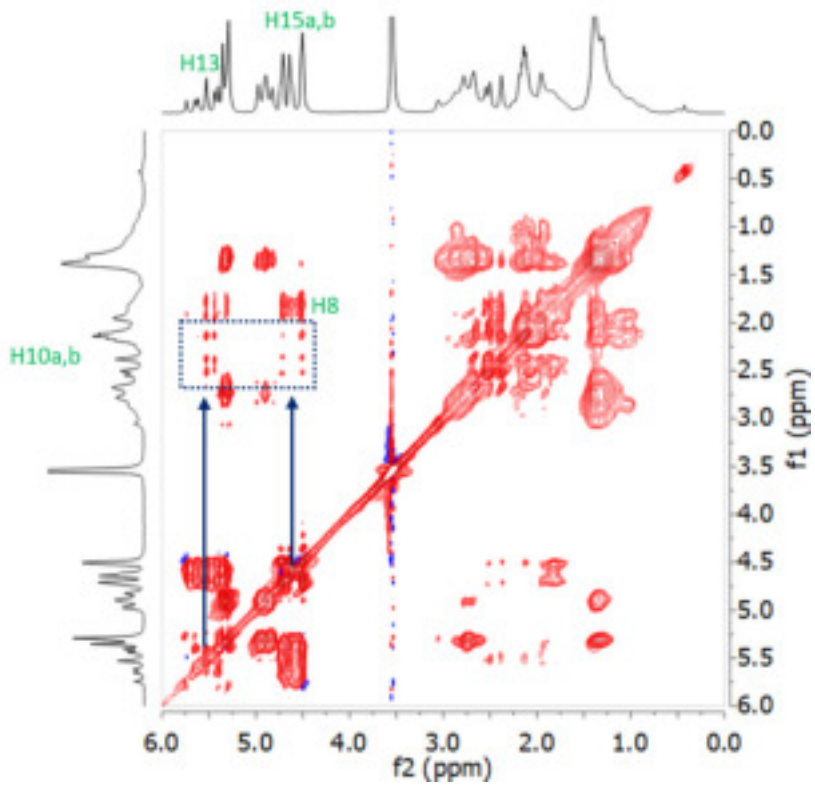

Figure 7. Region of the TOCSY spectrum of quinine. The experiment used a 150 ms spinlock

Like COSY, TOCSY correlates protons through chemical bonds. However, TOCSY can correlate protons over more bonds than COSY, which can be very useful in identifying individual subunits within a molecule. It can also be useful in overlapped or crowded spectra, where correlations can be “pushed” out into free space in spectrum. In the case of quinine, TOCSY provides additional confirmation of several assignments already made and, when combined with HSQC, allows the new assignment of H10a,10b through correlations from the alkene H13a, H15a, H15b, and confirmation correlation to H8a.

ROESY Spectrum

Figure 8 shows a region of the ROESY spectrum of quinine.

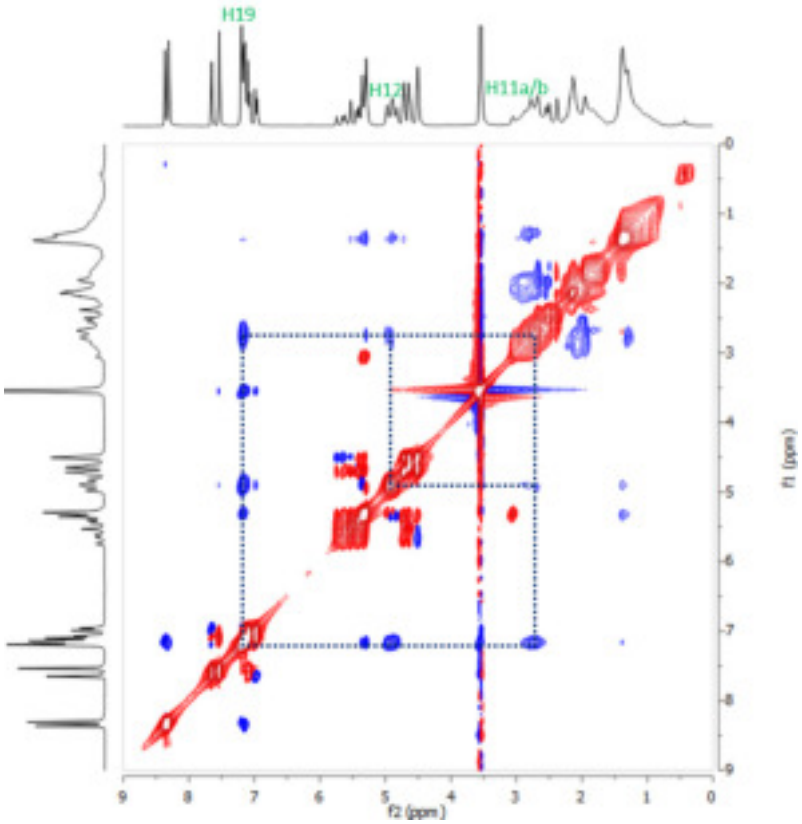

Figure 8. ^{1}H-^{1}H ROESY spectrum of quinine. The experiment used a 200 ms spinlock

In contrast to COSY and TOCSY, ROESY (and the related NOESY) provide “through-space” correlations between protons. This can be extremely useful in understanding the stereochemistry or conformation of a molecule, but it can also be useful in peak assignment. The ROESY spectrum of quinine shows strong correlations from H19 and H12 to an unassigned methylene at 2.85 ppm. From the known conformation of quinine, this peak must be due to H11a or H11b.

Complete Assignment

Table 2 below shows the complete ^{1}H and ^{13}C peak assignments for quinine.

Atom	1H (ppm)	^{13}C (ppm)
1	5.33	...
2	...	...
3	...	...
4	...	...
5	1.33	27.6
6	2.73	60.8
7	1.3	24.2
8	1.8	39.7
9	1.1	27.6
10	2.3	55.6
11	2.8, 2.15	41.9
12	4.9	71.2
13	5.6	142.7
14	...	149.4
15	4.6	114.1
16	...	127.2
17	...	119.2
18	...	144.0
19	7.2	102.6
20	8.3	147.6
21	7.6	131.3
22	...	157.3
23	7.0	121.0
24	3.6	55.6

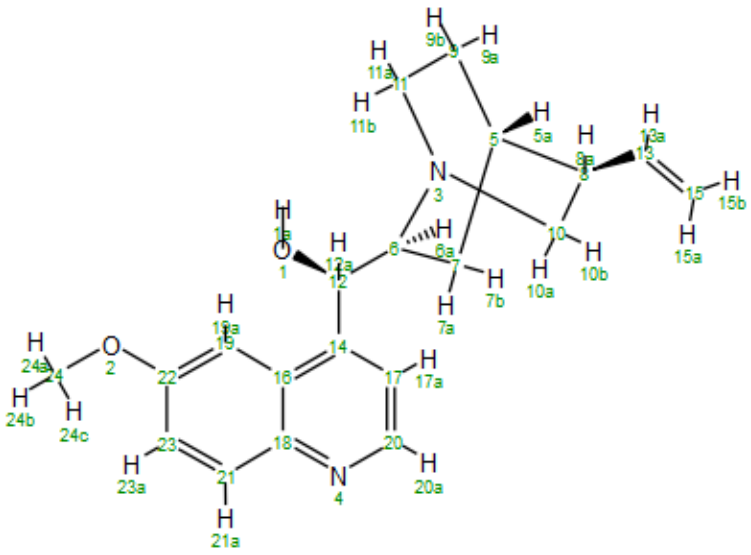

Table 2. Complete ^{1}H and ^{13}C peak assignments of quinine

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Conclusions

It has been shown how, through the application of 1D and 2D NMR methods, verification of the structure of quinine via the unambiguous assignment of its ^{1}H and ^{13}C peaks, can be performed using a Spinsolve 80 spectrometer. Furthermore, the accuracy of these assignments was confirmed by referring to a similar analysis carried using a high-field NMR system. [2]

References

1. WHO's List of Essential Medicines:
   http://www.who.int/medicines/publications/essentialmedicines/en/

2. "Assignment Strategies Using Modern NMR Methods: Quinine in benzene-d6":
   https://www.chem.wisc.edu/~cic/nmr/NMRdatab/res_cmpd/pdfs/quin_assignment_example-for-pdf.pdf