NMR hide-and-seek challenge

Reinhard Meusinger¹

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We would like to invite you to participate in the Analytical Challenge, a series of puzzles to entertain and challenge our readers. This special feature of “Analytical and Bioanalytical Chemistry” has established itself as a truly unique quiz series, with a new scientific puzzle published every other month. Readers can access the complete collection of published problems with their solutions on the ABC homepage at <http://www.springer.com/abc>. Test your knowledge and tease your wits in diverse areas of analytical and bioanalytical chemistry by viewing this collection.

In the present challenge, NMR is the topic. And please note that there is a prize to be won (a Springer book of your choice up to a value of €100). Please read on...

Meet the challenge

In contrast to the analyst who interprets spectra, children love to play hide-and-seek. If they play outdoors, they may have inadvertent exposure to the substance that is the object of this challenge. The substance we are looking for in this challenge is found in small amounts in our own bodies and it is also found in the nettle hair of the common stinging nettle (*Urtica dioica*,

Fig. 1). The compound was synthesized first by two German chemists at the beginning of the twentieth century as a chemical curiosity. Three years later the compound was observed in plants and humans, and its physiological function and biosynthesis had already been studied by then.

Scientists performing structural identification of compounds are sometimes faced with peculiar uncertainties. Particularly, this concerns the incompleteness of the observed signals in the experimental spectra. So, this challenge represents a hide-and-seek game caused by hidden NMR signals.

First, consider the ¹H NMR spectrum of our substance. Depending on the solvent, the expected number of signals in this first-order spectrum of the small molecule with the molar mass of 111.2 g/mol alternates between six in DMSO-*d*₆ and four in D₂O. Surprisingly, the experimental spectrum, measured in dry DMSO-*d*₆ followed by the subsequent automatic processing, shows only four signals with a relative ratio of intensities of 1H:1H:2H:2H (Fig. 2, top). However, when the spectrum was manually phased and baseline corrected, a further extremely broad signal near 4 ppm was uncovered (Fig. 2, bottom). Its relative intensity of 3H was determined by the difference of the integrals in the region from 2 to 8 ppm of these two spectra from the same measurement. There are several effects that can cause line broadening in liquid NMR spectroscopy. Here, it is being caused by dynamic processes such as proton exchange reactions between the exchangeable hydrogen atoms which occur during the relatively long NMR acquisition time. In the worst case scenario, the involved signals are extremely wide and their

✉ Reinhard Meusinger
meusi@nmr.chemie.tu-darmstadt.de

¹ Institute of Organic Chemistry and Biochemistry, University of Technology Darmstadt, Alarich-Weiss-Str. 4, D-64287 Darmstadt, Germany

Fig. 1 A few nanograms of the compound were found in the nettle hair of the common stinging nettle (*Urtica dioica*)

intensity is entirely hidden in the baseline as shown in the lower spectrum in Fig. 2.

Even larger problems arise in the evaluation of ^{13}C NMR spectra (Fig. 3). Only three substance-derived signals were observed here with a narrow line width. The two high-field shifted signals were identified as methylene carbon atoms

from DEPT-135 spectrum (Fig. 3, top). Another much more broadened signal at 117.7 ppm was not observed in the DEPT-135 spectrum and could therefore be identified as a quaternary carbon. The heteronuclear single bond correlation (HSQC) spectrum, indicating direct coupling between proton and carbon ($^1J_{\text{H-C}}$), however, shows that this signal represents a CH carbon and an additional sharp signal at 134.4 ppm (Fig. 4, top left). Now consider the multiple bond correlation (HMBC) spectrum which indicates couplings over two ($^2J_{\text{H-C-C}}$) and/or three ($^3J_{\text{H-C-C-C}}$) covalent bonds. Cross peaks were detected for the carbon signal at 134.4 ppm all with narrow ^1H signals, including the directly bonded hydrogen atom at 7.5 ppm (Fig. 4, bottom left). Possibly, the carbon signal at 134.4 ppm is therefore a matter of a further hidden carbon either induced by molecular symmetry or by two randomly isochronal signals. However, the digital spectral resolution of 0.3 Hz/point achieved here by a supplementary zero filling option of the experimental free induction decay is insufficient for an unambiguous differentiation (see enlarged signal in Fig. 3). The reasonable suspicion is confirmed by a quantitative ^{13}C NMR spectrum. Therefore, the inverse gated decoupling technique and a long repetition time (>10 s) were considered. By means of these conditions and after 15 h measurement time, a nearly quantitative interpretable ^{13}C NMR spectrum was obtained (see integrals in Fig. 3). Now it becomes obvious that the signal at 134.4 ppm is caused by two carbon atoms. After the complete listing of hydrogen and carbon signals, the assignment of the cross peaks in the HMBC spectrum still remains difficult. Therefore, a second set of spectra was measured from the dihydrochloride of the compound. Now, all the hidden signals in ^1H and ^{13}C NMR spectra are visible as shown in the projections of the HSQC and HMBC

Fig. 2 500 MHz ^1H NMR spectra of the same measurement in $\text{DMSO}-d_6$ (ref. 2.5 ppm) with (top) and without (bottom) automatic processing including phase and baseline corrections. The broad signal around 4 ppm disappears during the automatic baseline correction

Fig. 3 125 MHz ^{13}C NMR distortionless enhanced polarization transfer DEPT-135 (*top*) spectra with respect to DMSO- d_6 at 39.5 ppm and quantitative ^{13}C NMR spectrum measured in 15 h with inverse gated decoupling and a long repetition time (*bottom*). While no shift difference is observed for the signal at 134.4 ppm with best possible spectrum resolution (*inset*) a further hidden carbon signal is indicated here by the double intensity of this signal

spectra of this compound in Fig. 4 (right column). All two-dimensional spectra in Fig. 4 were pictured with comparable scaling. Furthermore, the comparable carbon signals are

connected by dotted artificial lines. Now, the random isochronism of both signals of a CH and a quaternary carbon at 134.4 ppm is shown by switchover from lower left to the

Fig. 4 ^{13}C heteronuclear single bond (HSQC, *top*) and multiple bond (HMBC, *bottom*) correlation spectra of the pure compound (*left column*) and of the dihydrochloride (*right column*). All spectra were measured in DMSO- d_6 and were comparable detruncated for the best resolution in both dimensions

Fig. 5 Infrared spectrum of the dihydrochloride (KBr)

Fig. 6 Electron impact mass spectrum of the dihydrochloride

lower right spectrum, quite clearly. Finally, the infrared spectrum (Fig. 5) and the electron impact mass spectrum (Fig. 6) of the dihydrochloride of this natural product are provided for structural analysis purposes.

Can you identify this substance from its peculiar NMR spectra?

We invite our readers to participate in the Analytical Challenge by solving the puzzle above. Please send the correct solution to abc-challenge@springer.com by December 1, 2016. Make sure you enter “NMR hide-and-seek challenge”

in the subject line of your e-mail. The winner will be notified by e-mail and his/her name will be published on the “Analytical and Bioanalytical Chemistry” homepage at <http://www.springer.com/abc> and in the journal (volume 409 /issue10) where readers will find the solution and a short explanation.

The next Analytical Challenge will be published in 409/1, January 2017. If you have enjoyed solving this Analytical Challenge you are invited to try the previous puzzles on the ABC homepage.