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Deconvolution of Complex NMR Spectra in Small Molecules by Multi Frequency Homonuclear Decoupling (MDEC)

Ana Paula D. M. Espindola,[†] Ronald Crouch,[‡] John R. DeBergh,[†] Joseph M. Ready,[†] and John B. MacMillan*,[†]

Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75390-9038, and Applications Laboratory, Varian, Inc., Palo Alto, California 94303

Received August 22, 2009; E-mail: john.macmillan@utsouthwestern.edu

Advances in NMR instrumentation have pushed the limits of small molecule NMR, with higher fields allowing better signal dispersion and modern probe design permitting analysis[1] and quantification[2] on the nanomolar level. One of the remaining limitations of small molecule NMR is the ability to obtain relative configuration of small molecules. Since the 1959 report by Karplus[3] the use of coupling constants to determine the relative configuration in conformationally rigid small molecules has become routine. More recently, *J*-based configuration analysis[4] has been applied to determine the relative stereochemistry of complex acyclic and macrocylic small molecules and natural products.[5] The utility of *J*-based methods relies on the ability to measure discrete coupling constants between protons (J_{HH}), which can be difficult in molecules with complex multiplets and significant signal overlap. The existing methods, such as E.COSY[6] and 2D *J*-resolved, suffer from lack of sensitivity and complex data analysis. Alternatively, classic homonuclear decoupling can be used to simplify a complex multiplet but is limited to irradiating a single proton.[7] Due to the challenges of measuring coupling constants, many reports of natural products and synthetic small molecules do not report vital coupling information; rather they only assign signals as multiplets. In this paper we report an experiment with selective homonuclear decoupling of multiple protons simultaneously that allows a fast and reliable determination of specific coupling values from complex spectra.

The development of this experiment was inspired by our studies to determine the relative stereochemistry of unsaturated fatty acids in which we were faced with complications due to multiple $^3J_{HH}$ and allylic couplings, making assignment of individual coupling constants difficult. Advances in NMR technology allowed us to utilize a q3 shaped pulse to obtain a multi frequency homodecoupling (MDEC) during the acquisition time. In combination with the standard ^{1}H experiment, it results in a complete simplification of a complex multiplet. The usefulness of our MDEC experiment is demonstrated with three compounds of increasing structural and spectral complexity: menthol (**1**), cholesteryl acetate (**2**), and a synthetic C_{16} fatty acid (**3**) (Figure 1).

As proof of principle, menthol (**1**) offers a ^{1}H NMR spectrum that is well resolved but has complex multiplets. In particular, the proton H-2 (δ 0.99 ppm) appears as a dddd, due to coupling to four distinct protons (Figure 2a).[8] Application of homonuclear decoupling with a hard pulse at H-1 (δ 3.25 ppm) causes H-2 to collapse to an apparent doublet of triplets (Figure 2b). Application of a selective q3 shaped pulse to irradiate protons H-1 and H-7 (δ 2.11 ppm) results in the H-2 multiplet collapsing to a distinct doublet of doublets with coupling to H-3b ($^3J_{HH}$ = 12.2 Hz) and H-3a ($^3J_{HH}$ = 3.3 Hz) (Figure 2c).[9] Additional decoupling of H-3a (δ 1.49 ppm) collapses H-2 to a doublet with a 12.2 Hz coupling (Figure 2d). An advantage of this experiment is the sensitivity, requiring only eight scans (60 s) for a 20 mM solution of menthol. In a matter of minutes, it is possible to reliably ascertain all $^3J_{HH}$ from a complex multiplet. This compares to an E.COSY (Supporting Information Figure 6), which required multiple hours to obtain sufficient *S*/*N* and resolution to reliably measure coupling constants. It should be noted that E.COSY offers the advantage of analyzing all coupling information for a molecule in a single experiment, although due to optimization parameters this may require multiple experiments for complex small molecules.[10]

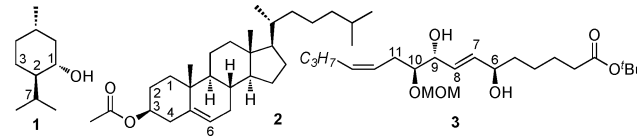

Figure 1. Structure of compounds used for the MDEC experiments.

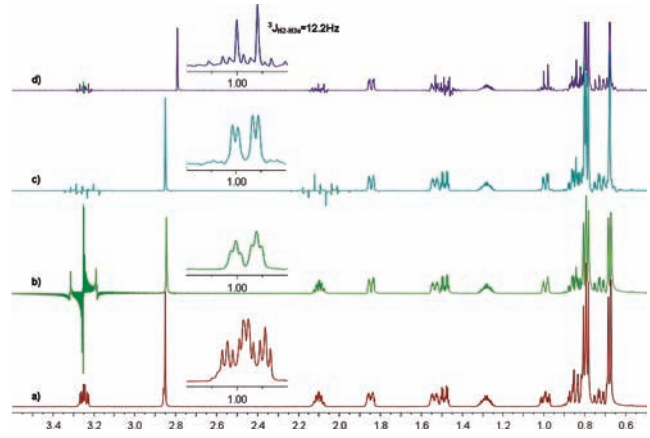

Figure 2. Menthol (**1**) ^{1}H NMR data in $CDCl_3$. (a) ^{1}H NMR of **1**. (b) Homonuclear decoupling of **1** with irradiation at H-1. (c) MDEC irradiation of **1** at H-1 and H-8. (d) MDEC irradiation of **1** at H-1, H-3a, and H-8.

Cholesteryl acetate (**2**) offers a more complex spectral pattern, due to the overlapping methylenes that comprise the tetracylic ring system. The ^{1}H NMR region from δ 1.40−1.60 ppm is comprised of four overlapping multiplets that prohibit reliable measurements of coupling constants (Figure 3a). Application of the MDEC methodology alone would not provide sufficient deconvolution of the signals; however, MDEC can be incorporated in other selective excitation 1D experiments such as a 1D-TOCSY to isolate individual spin systems prior to decoupling.[11] A selective 1D-TOCSY of **2** by irradiation of H-3 (δ 4.57 ppm) results in the isolation of the spin system from H-1 through H-4 (Figure 3b).

[†] University of Texas Southwestern Medical Center at Dallas.
[‡] Varian, Inc.

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The signal associated with H-2a (δ 1.56 ppm), obscured in the standard ^{1}H experiment, can be resolved to a complex multiplet in the 1D-TOCSY experiment. The use of a selective 1D-TOCSY-MDEC experiment with initial selection of H-3 for the TOCSY followed by decoupling of H-3, H-2b, and H-1b results in the subsequent collapse of the H-2a mutliplet to a doublet with a coupling constant of 13.7 Hz, consistent with the trans-diaxial relationship between H-1a and H-2a (Figure 3c).

Recently, the potent cytotoxic natural product nigricanoside A was reported with only a planar structure. The small quantity of material and the overlapping ^{1}H NMR precluded assignment of the relative configuration of the C_{16} and C_{20} fatty components.[12] Our efforts toward assigning the relative stereochemistry via NMR studies of synthetic models were hindered by the inability to assign the proton coupling constants of interest. We applied a combination of MDEC and the 1D-TOCSY-MDEC to obtain the necessary coupling information from the synthetic C_{16} fatty acid **3** to assign the C9/C10 *anti* stereochemistry. The key coupling values were obtained from deconvolution of H-9 (δ 3.77 ppm). Systematic decoupling of two protons simultaneously resulted in the assignment of $^3J_{H9-H10} = 3.5$ Hz, $^3J_{H10-H11a} = 4.7$ Hz, and $^3J_{H10-H11b} = 7.4$ Hz (Figure 4b−d). Further stereochemical and synthetic studies are underway to assign the relative configuration of **3** and its diastereomers.[13]

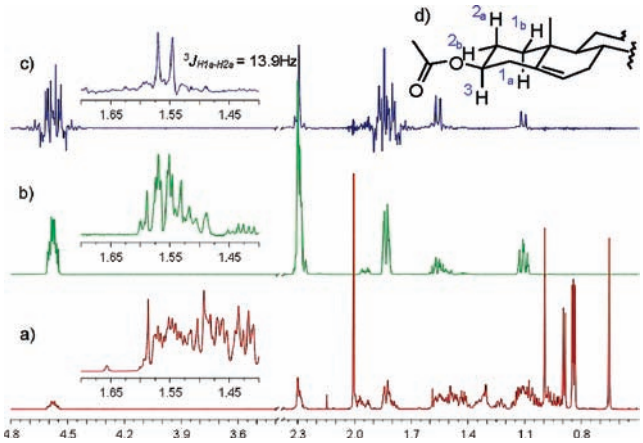

Figure 3. (a) ^{1}H NMR of **2** in $CDCl_3$. (b) 1D-TOCSY of **2** with selection of H-3. (c) 1D-TOCSY with selection of H-3 followed by simultaneous irradiation at H-1b, H-2b, and H-3. (d) A-ring of cholesteryl acetate (**2**).

Our experiments started with the selection of a shaped pulse. The q3 Gaussian was selected to invert the signal of interest, as it efficiently irradiates the peak of interest but with little net effect outside the chosen bandwidth, resulting in highly selective irradiation.[14] In all of our experiments, we gave preference to a small duty cycle (between 0.02 to 0.05) and higher decoupling power and applied a correction for the Bloch−Siegert effect in our multiple band excitation. This correction is necessary to account for small changes in the chemical shift due to the closeness of the irradiation frequency and the resonance frequency.[15,16]

The MDEC experiment fills a gap in the current methods to measure proton homonuclear coupling constants. In a matter of a few minutes complex multiplets can be simplified to easily interpretable doublets. In comparison with the E.COSY experiment, MDEC is faster, more sensitive, and easier to interpret. This is especially the case when only a few coupling values are necessary. Additionally, the single pulse MDEC experiment can be incorporated in other 1D experiments, increasing its power and allowing a better tailoring of the experiments to solve specific problems. If desired the sites where MDEC was applied could be effectively removed from the spectra by presaturation with the same waveform combined with a two-step phase cycle.

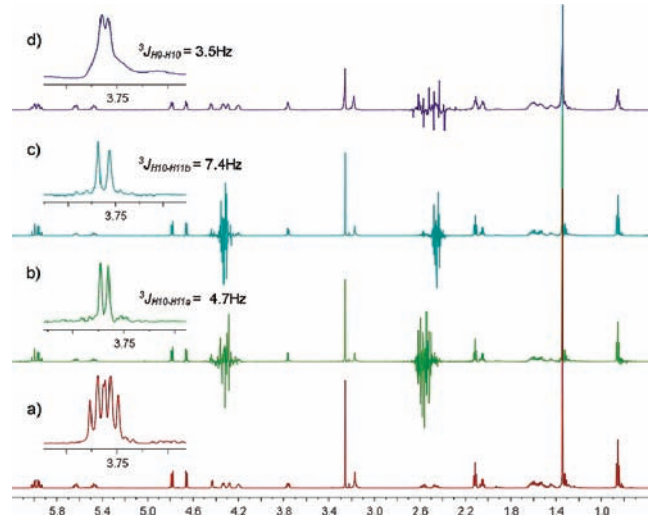

Figure 4. (a) ^{1}H NMR of **3** in 25:2 C_6D_6/DMSO-d_6. (b) Decoupling at H-9, H-11b. (c) Decoupling at H-9, H-11a. (d) Decoupling at H-11a, H11b.

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Supporting Information Available: Experimental procedures, representative NMR spectra, and experiment macros. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Olson, D. L.; Norcross, J. A.; O'Neil-Johnson, M.; Molitor, P. F.; Detlefsen, D. J.; Wilson, A. G.; Peck, T. L. *Anal. Chem.* **2004**, *76*, 2966–2974.
(2) Dalisay, D. S.; Tsukamoto, S.; Molinski, T. F. *J. Nat. Prod.* **2009**, *72*, 353–359.
(3) (a) Karplus, M. *J. Chem. Phys.* **1959**, *30*, 11–15. (b) Karplus, M. *J. Chem. Phys.* **1959**, *30*, 15–18.
(4) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866–876.
(5) For examples see; (a) Williamson, R. T.; Boulanger, A.; Vulpanovici, A.; Roberts, M. A.; Gerwick, W. H. *J. Org. Chem.* **2002**, *67*, 7927–7936. (b) Cimino, P.; Bifulco, G.; Evidente, A.; Abouzeid, M.; Riccio, R.; Gomez-Paloma, L. *Org. Lett.* **2002**, *4*, 2779z–2782.
(6) Griesinger, C.; Sorensen, O. W.; Ernst, R. R. *J. Chem. Phys.* **1986**, *85*, 6837–6852.
(7) Claridge, T. D. W. *High-Resolution NMR Techniques in Organic Chemistry*; Pergamon: New York, 1999.
(8) The resolution of the multiplet at δ 0.99 ppm is sufficient to measure the four coupling constants. We chose this signal as a clear example of how the MDEC experiment can reduce the complexity.
(9) Kwan, E. E.; Huang, S. G. *Eur. J. Org. Chem.* **2008**, *267*, 1–2688.
(10) Griesinger, C.; Sorensen, O. W.; Ernst, R. R. *J. Magn. Resonan.* **1987**, *75*, 474–492.
(11) For selective excitation experiments in natural products, see: (a) Morinaka, B. I.; Pawlik, J. R.; Molinski, T. F. *J. Nat. Prod.* **2009**, *72*, 259–64. (b) Vidal, P.; Esturau, N.; Parella, T.; Espinosa, J. F. *J. Org. Chem.* **2007**, *72*, 3166–3170.
(12) Williams, D. E.; Sturgeon, C. M.; Roberge, M.; Andersen, R. J. *J. Am. Chem. Soc.* **2007**, *129*, 5822–5823.
(13) The synthetic and stereochemical analysis of the C_{16} fatty acids and their relationship to nigricanoside A will be published in due course.
(14) Emsley, L.; Bodenhausen, G. *J. Magn. Reson.* **1992**, *97*, 135–138.
(15) (a) Kupce, E.; Freeman, R. *J. Magn. Reson., Ser. A* **1995**, *112*, 261–264. (b) Mersh, J. D.; Sanders, J. K. M. *J. Magn. Reson.* **1982**, *50*, 289–298.
(16) A stepwise procedure for acquiring the MDEC experiment and the 1D-TOCSY MDEC experiment is included in the Supporting Information.

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