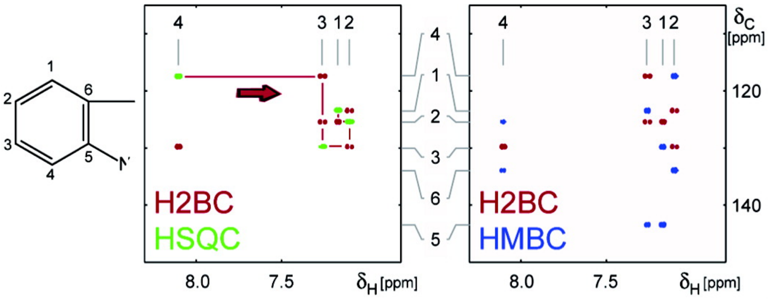
Communication

Heteronuclear Two-Bond Correlation: Suppressing Heteronuclear Three-Bond or Higher NMR Correlations while Enhancing Two-Bond Correlations Even for Vanishing *J*

Nils T. Nyberg, Jens . Duus, and Ole W. Srensen

J. Am. Chem. Soc., **2005**, 127 (17), 6154-6155• DOI: 10.1021/ja050878w • Publication Date (Web): 09 April 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 13 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
COMMUNICATIONS

Heteronuclear Two-Bond Correlation: Suppressing Heteronuclear Three-Bond or Higher NMR Correlations while Enhancing Two-Bond Correlations Even for Vanishing $^2J_{CH}$

Nils T. Nyberg, Jens Ø. Duus, and Ole W. Sørensen*

Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark

Received February 10, 2005; E-mail: olews@crc.dk

In the small-molecule NMR of natural isotopic abundance samples, the two-dimensional (2D) HMBC experiment[1] stands out as one of the key NMR experiments in structure determination. While the basic three-pulse multiple-quantum element has been retained over the years, the pulse sequence has been refined to include enhanced low-pass J filters, gradients,[2] and a scheme for uniform excitation over a range of heteronuclear long-range coupling constants.[3] HMBC spectra provide a wealth of correlations over multiple bonds, and while this on one hand is desirable, it poses the problem of distinguishing particularly between two- and three-bond correlations. Provided that ^1H spectral crowding is not excessive, a COSY spectrum is often used to alleviate that problem, but the much better spectral dispersion offered by, for example, ^{13}C, makes it preferable to be able to differentiate two- and three-bond correlations directly in an HMBC-type 2D spectrum.

We report a novel experiment, H2BC (Figure 1), leading to such an HMBC-type spectrum almost exclusively showing only two-bond correlations and markedly also two-bond correlations that are absent in HMBC spectra. Conceptually, H2BC can be understood as an HMQC−COSY combination[4] tailored to the needs of small-molecule heteronuclear NMR spectroscopy. A low-pass J filter[5,6] suppresses one-bond correlations after coherence transfer from ^{13}C-attached protons to non-^{13}C-attached protons.

The new H2BC experiment relies on $^1J_{CH}$ and $^nJ_{HH}$ couplings for coherence transfer and is fully independent of long-range $^nJ_{CH}$ couplings, that is, the HMBC amplitude factor $\sin(\pi\, ^nJ_{CH}\Delta)$, where Δ is the usual HMBC-type excitation delay, is absent from the peak intensity expressions. Furthermore, heteronuclear decoupling is employed during acquisition, which compares favorably with HMBC where either the peaks are antiphase with respect to $^nJ_{CH}$ or an additional refocusing delay Δ is introduced contributing another factor $\sin(\pi\, ^nJ_{CH}\Delta)$ from refocusing $^nJ_{CH}$. A constant-time[7] setup suppresses all homo- and heteronuclear couplings in the indirect dimension of the experiment. Finally, other benefits over HMBC are that H2BC spectra exhibit pure absorption peak shapes and that the pulse sequence is significantly shorter.

The critical dependence on long-range coupling constants of the intensity expression for a peak between ^{13}C spin j and ^1H spin k is

$$I \propto \sin\{\pi J(H_jH_k)T\}\prod_{i\neq k}\cos\{\pi J(H_jH_i)T\} \qquad (1)$$

where the product of the cosines also includes possible geminal coupling constants, $^2J(H_jH_j)$. The setting of the constant-time delay T to build up coherence antiphase with respect to a single other proton k is a compromise between decreasing cosine factors and an increasing sine factor. In practice, we have found that apart from peaks associated with vanishing cosine factors in eq 1 or absence of $^2J_{HH}$, H2BC spectra are typically rather insensitive to T in the range $14 < T < 22$ ms. Finally, note that eq 1 is not symmetric with respect to permutation of indices j and k.

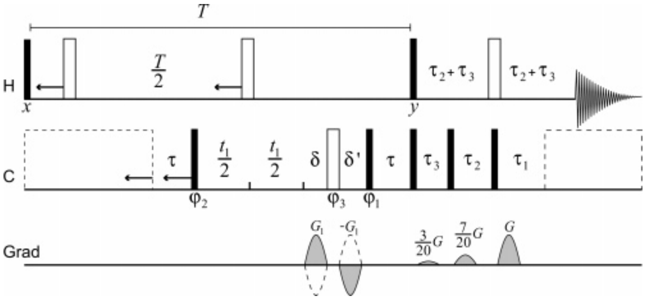


Figure 1. Pulse sequence H2BC with a 3rd order low-pass J filter for correlating ^1H and ^{13}C nuclei via $^1J_{CH}$ and $^1H-^1H$ couplings. Filled and open bars refer to $\pi/2$ and π pulses, respectively, while the dashed boxes represent ^{13}C decoupling. $\tau_1 = {}^1\!/_2\,[^1J_{min} + 0.07(^1J_{max} - {}^1J_{min})]^{-1}$, $\tau = \tau_2 = [^1J_{max} + {}^1J_{min}]^{-1}$, $\tau_3 = {}^1\!/_2\,[^1J_{max} - 0.07(^1J_{max} - {}^1J_{min})]^{-1}$, and $\delta' = \delta + t(\pi^H)$ where δ is the gradient delay. T denotes the constant-time delay. The gradient strength G_1 is G and $-G$ for echo and antiecho selection, respectively. The recommended phase cycle is $\varphi_1 = \{x, -x, -x, x\}$, $\varphi_2 = \{x, x, 4(-x), x, x\}$, $\varphi_3 = \{4(x), 4(y), 4(-x), 4(-y)\}$ with receiver phase $\{x, -x\}$.

H2BC spectra only show peaks involving protonated ^{13}C spins j, but the ^1H spin k of the correlation can be attached to any type of atom provided that $^3J(H_jH_k)$ does not vanish. The peaks are antiphase with respect to $^3J(H_jH_k)$ in the acquisition dimension.

The discrimination between two- and three-bond correlation in H2BC is based solely on the size of $^nJ_{HH}$ coupling constants, and as these do not uniformly vanish for $n > 3$, there is no absolute guarantee that a peak in an H2BC spectrum represents a two-bond correlation. However, as a rule of thumb, correlations that are strong in an H2BC spectrum and weak in an HMBC spectrum indicate two-bond correlations, while vice versa is indicative of a three-bond correlation. Possible remaining ambiguities associated with peaks of similar intensities in H2BC and HMBC must be resolved based on the clear two- and three-bond assignments.

In Figure 2, a comparison between an H2BC (Figure 2a) and an HMBC (Figure 2b) spectrum of strychnine recorded under identical conditions and using the same contour levels is shown. The spectra are quite complementary, as Figure 2a shows all possible two-bond correlations and only 3 three-bond peaks (C14/H22, C22/H14, and C3/H1) caused by nonvanishing $^4J(H^{14}-H^{22})$ and $^4J(H^1-H^3)$, while in Figure 2b, eight two-bond peaks are missing (indicated by boxes) and there are many three-bond correlations.

The almost exclusivity of two-bond correlations in H2BC makes it possible to extract INADEQUATE-type connectivity information by overlaying H2BC and HSQC spectra, as illustrated in Figure 3, with spectra of strychnine with the chains 1−4, 11(8)−16, and 17−18 marked.

The complementarity of HSQC, H2BC, and HMBC spectra could prove valuable for a semi-automated analysis of this package of spectra based on the H2BC−HMBC rule of thumb mentioned above and the unique one-bond correlations in HSQC.

10.1021/ja050878w CCC: $30.25

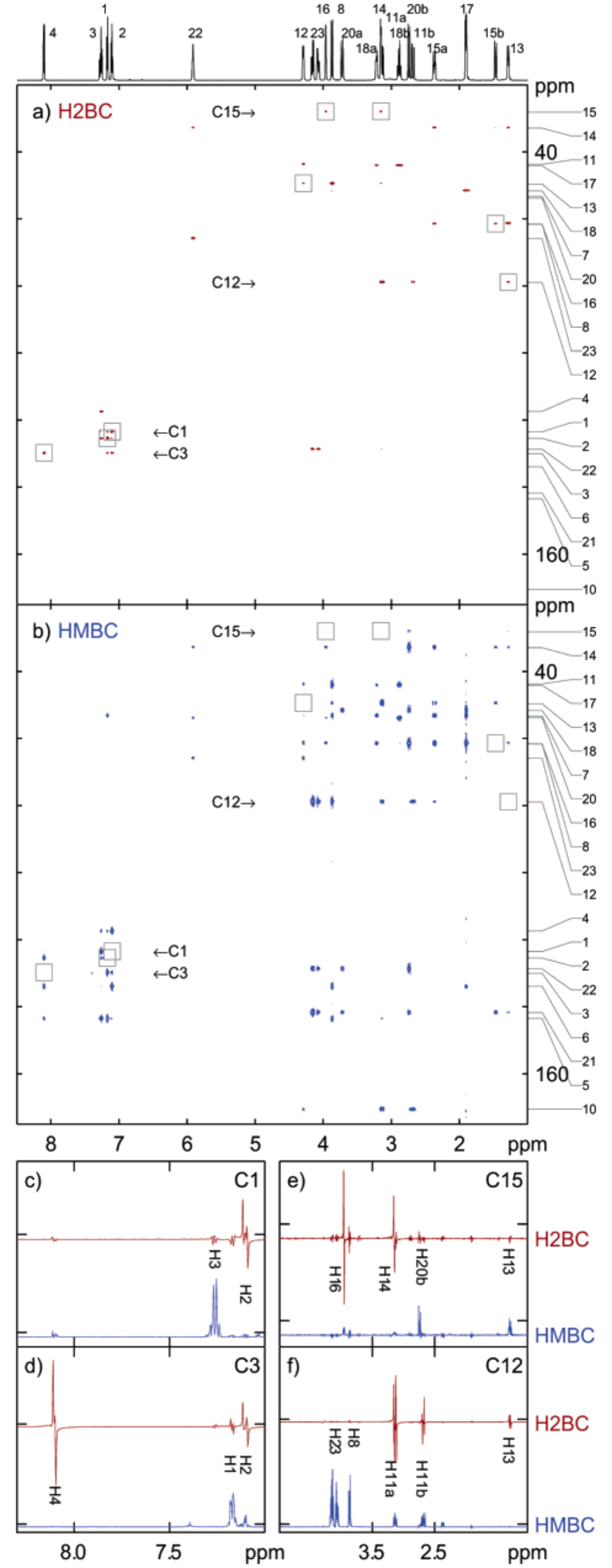


Figure 2. (a) H2BC ($T = 21.9$ ms) and (b) HMBC ($\Delta = 65$ ms) spectra of strychnine dissolved in $CDCl_3$ and recorded on a Bruker 600 MHz spectrometer with 3rd order low-pass J filters set for the range 125 Hz < ^1J < 170 Hz, data matrix (512, 2k) linear predicted to (1k, 4k) prior to 2D FT, cosine window function in t_1 and a $\pi/4$ shifted sine bell in t_2, relaxation delay 2 s. Boxes indicate the eight two-bond peaks missing in HMBC. (c−f) Selected sections through the two spectra at the positions of the ^{13}C nuclei indicated and with the lowest contour level employed in (a−b) marked on the vertical borders.

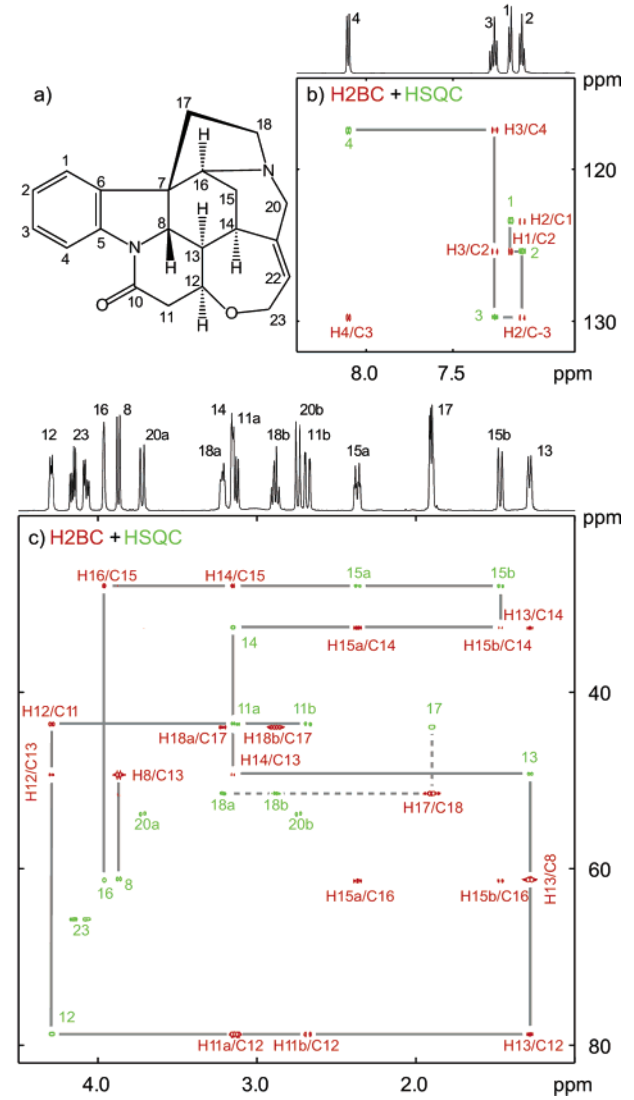


Figure 3. (a) Strychnine structure, (b−c) excerpts of overlaid HSQC (green) and H2BC (red, same data as in Figure 2) spectra of strychnine.

H2BC is not the first attempt at distinguishing two- and three-bond correlations in HMBC spectra.[8] However, earlier attempts based on the HMBC pulse sequence fail to enhance two-bond correlations.

In conclusion, we have introduced a new experiment for $^1H-^{13}C$ heteronuclear two-bond correlation that is highly complementary to HMBC and allows INADEQUATE-type walks requiring only molecules with a single ^{13}C nucleus.

References

(1) Bax, A.; Summers, M. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094.
(2) Willker, W.; Lebfritz, D.; Kerssebaum, R.; Bermel, W. *Magn. Reson. Chem.* **1993**, *31*, 287−292.
(3) (a) Meissner, A.; Sørensen, O. W. *Magn. Reson. Chem.* **2000**, *38*, 981−984. (b) Schulte-Herbrüggen, T.; Meissner, A.; Papanikos, A.; Meldal, M.; Sørensen, O. W. *J. Magn. Reson.* **2002**, *156*, 282−294.
(4) Griesinger, C.; Sørensen, O. W.; Ernst, R. R. *J. Magn. Reson.* **1989**, *84*, 14−63.
(5) Kogler, H.; Sørensen, O. W.; Bodenhausen, G.; Ernst, R. R. *J. Magn. Reson.* **1983**, *55*, 157−163.
(6) Sørensen, O. W.; Dønstrup, S.; Bildsøe, H.; Jakobsen, H. J. *J. Magn. Reson.* **1983**, *55*, 347−354.
(7) Bax, A.; Mehlkopf, A. F.; Smidt, J. *J. Magn. Reson.* **1979**, *35*, 167−169.
(8) Sprang, T.; Bigler, P. *Magn. Reson. Chem.* **2003**, *41*, 177−182 and references therein.

JA050878W