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Experimental and Theoretical Investigation of $^{1}J_{CC}$ and $^{n}J_{CC}$ Coupling Constants in Strychnine

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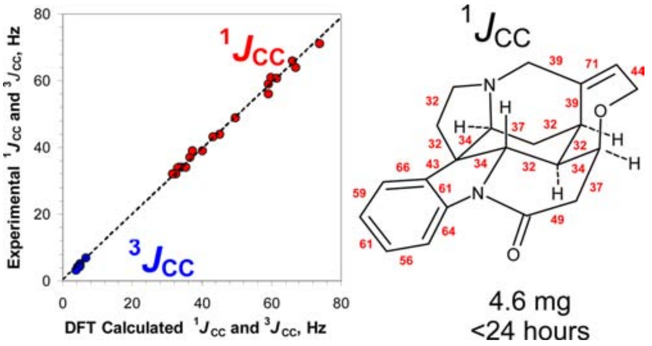
ABSTRACT



A relatively unexplored and unexploited means of establishing molecular structure, stereochemistry, and probing vicinal bond angles is through the use of long-range $^{13}C-^{13}C$ coupling constants. The measurement of these multifunctional, diagnostic $^{3}J_{CC}$ couplings has not been reported on sample amounts viable for the practicing organic chemist. A generalized protocol for the measurement of $^{1}J_{CC}$ and $^{3}J_{CC}$ couplings using a 4.6 mg sample of strychnine as a model compound is described, and the utility of DFT calculations for the prediction of these useful molecular descriptors and the congruence of the calculated and experimental data is demonstrated.

Numerous methods can be applied to determine the stereochemical orientation of substituents in organic molecules. Classically, proton–proton homonuclear coupling constants and nuclear Overhauser effect measurements have been used as the primary probes for this spatial information.[1] However, as molecular complexity increases and the number of protons in the vicinity of a stereocenter diminishes, the challenge of establishing the relative stereochemical configuration of a molecule increases dramatically.

Recently, more advanced methods such as the *J*-based configuration analysis method[2] have been developed to leverage the Karplus-type relationship[3] of $^{3}J_{CH}$ coupling constants as additional parameters when dealing with increasingly complex systems for stereochemical assignment purposes. To this end, a number of NMR pulse sequences have been developed to facilitate the measurement of these $^{2,3}J_{CH}$ heteronuclear couplings.[4]

Of these experiments, the HSQMBC[4,5] experiments have perhaps made the most significant impact to these studies and this sequence has proven to be exceedingly

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powerful in cases where there is at least one proton on each stereocenter of interest. In cases where there are too few protons in the system under examination, the analyst was, until now, destined to resort to alternate techniques such as single crystal X-ray crystallography (SCXRD),[6] or residual dipolar couplings (RDC)[7] in tandem with DFT calculations to determine the configuration of the molecule under study.[8]

It has been known for quite some time that $^3J_{CC}$ coupling constants also follow a Karplus-type relationship.[9] However, the measurement of these parameters has generally proved to be too daunting of a task for routine use on the size of samples typically available to the laboratory-based organic or natural products chemist. The fact that the magnitude of these $^nJ_{CC}$ couplings is highly dependent on a number of factors including substituent effects and bond angles has complicated matters further.[9] As a result, the unpredictability of the values of these coupling constants previously made them a less-than attractive option for general use since, in the past, the expected values could not be reliably predicted without a model system for comparison. Because of these hurdles, the routine use of $^{13}C-^{13}C$ coupling constants for the investigation of small molecule structure problems has been reported only occasionally in the literature and usually on molar or millimolar solutions in 5 mm or larger NMR tubes.[10]

In this work, we demonstrate that the measurement and reliable calculation of $^1J_{CC}$ and $^3J_{CC}$ coupling constants for samples smaller than 15 μmol can be easily obtained in reasonable amounts of time and that the expected values for these homonuclear $^{13}C-^{13}C$ coupling constants can be accurately calculated with state-of-the-art molecular modeling capabilities. The application of this powerful tool for the investigation of molecular structure is demonstrated here on strychnine (Figures 1, 2, and 5). Strychnine is a universally available and convenient model compound for the development, implementation, and testing of a wide variety of NMR experiments, and it has been used by various authors for the past several decades. The molecule has a diverse assortment of proton and carbon resonances types, reasonable chemical shift dispersion, and excellent solubility in deuterochloroform (1 g/6.5 mL). Recently, we have used strychnine as a model compound for the investigation and development of new hyphenated data presentations including HSQC-1,1-ADEQUATE and HSQC-1,n-ADEQUATE.[11] Of necessity, these studies required the acquisition of 1,1- and 1,n-ADEQUATE spectra to input into the covariance processing algorithms.[12]

Guidelines for the acquisition of 1,1-ADEQUATE spectra suggest that the range of $^1J_{CC}$ coupling constants is nominally 40−60 Hz and that 50 Hz represents a usable compromise optimization of the delays in the pulse sequence that are dependent on the $^1J_{CC}$ coupling. Our experience has shown that acquisition of 1,n-ADEQUATE spectra with a combination of delays set for 50 Hz and 5−7 Hz for $^1J_{CC}$ or $^nJ_{CC}$ couplings, respectively, represent a generalized best case scenario for optimization. In the case of strychnine, a SciFinder search of the chemical literature confirmed our suspicions that the $^1J_{CC}$ or $^nJ_{CC}$ couplings had never been measured.

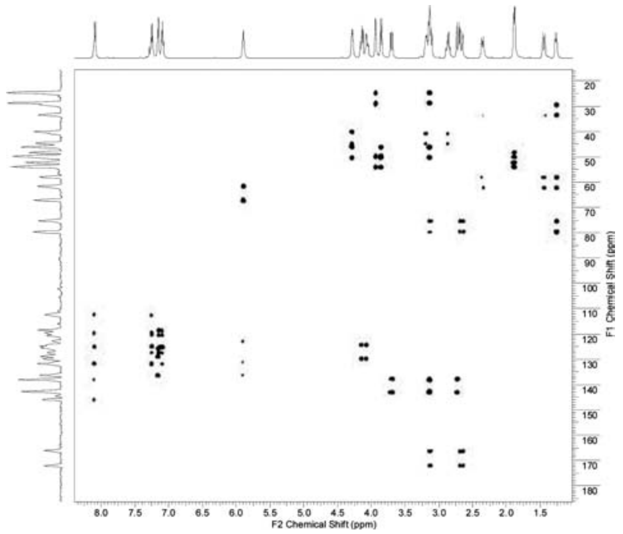

Figure 1. $^1J_{CC}$ *J*-modulated ADEQUATE spectrum acquired using a 4.6 mg sample of strychnine dissolved in 30 μL of deuterochloroform using a Bruker 600 MHz instrument equipped with a 1.7 mm TXI $^1H/^{13}C-^{15}N$ cryoprobe. These data were acquired in 23 h as 2048 × 200 points with 128 accumulated transients/t_1 increment with the scaling factor set to 15 and an F_1 spectral window of 180 ppm. Data were processed using linear prediction to 512 points in F_1 and zero-filling prior to apodization to afford a final data matrix that was 2048 points square.

J-Modulated ADEQUATE was developed as a tool to conveniently measure $^1J_{CC}$ coupling constants using a

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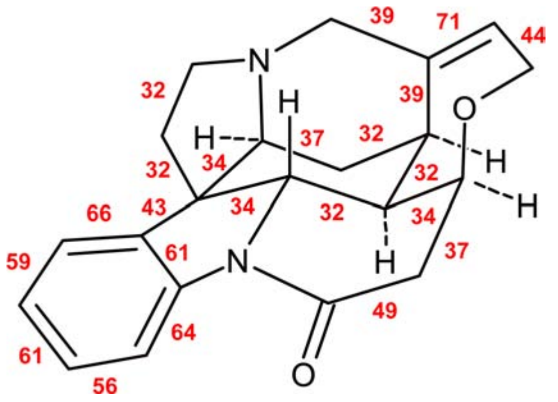

Figure 2. Structure of strychnine showing the ${}^{1}J_{CC}$ coupling constants measured by extracting slices from the ${}^{1}J_{CC}$ *J*-modulated ADEQUATE spectrum shown in Figure 1.

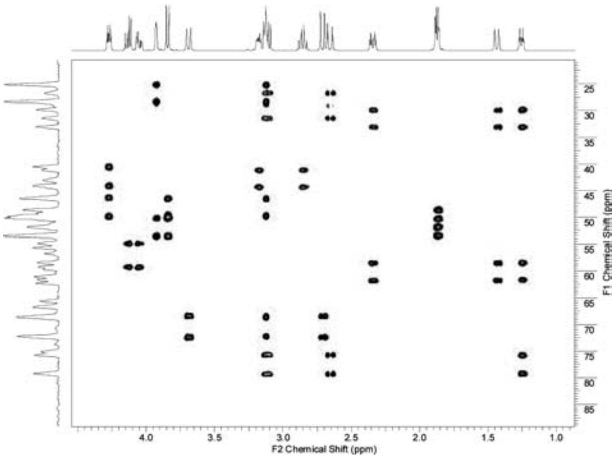

Figure 3. Expansion of the aliphatic region from the ${}^{1}J_{CC}$ *J*-modulated ADEQUATE spectrum shown in Figure 1.

scaling factor to mitigate F_1 digital resolution limitations.[13,14] It is on this basis that we now wish to report the measured ${}^{1}J_{CC}$ coupling constants of strychnine that range from 32 to 71 Hz and the ${}^{3}J_{CC}$ coupling constants that range from 3.1 to 7.0 Hz. In this study, we demonstrate today's modern cryogenically cooled NMR probes allow detection and measurement of reliable values of ${}^{1}J_{CC}$ and ${}^{n}J_{CC}$ NMR coupling constants on samples as small 13.8 μmol (4.6 mg strychnine). We have also embarked on a study to compare calculated values of ${}^{1}J_{CC}$ and ${}^{n}J_{CC}$ to those measured experimentally.

The first phase of this study was to measure the previously unreported ${}^{1}J_{CC}$ coupling constants of strychnine. To facilitate extraction of these coupling constants, we employed the *J*-modulated 1,1-ADEQUATE experiment proposed by Kövér and co-workers[13] and further refined by Bermel and Thiele.[14]

The spectral data for the ${}^{1}J_{CC}$ couplings are shown in Figures 1 and 3, and the measured couplings are shown superimposed on the structure in Figure 2. In the case of the ${}^{1}J_{CC}$ couplings, we determined that any scaling factor over 12 allowed accurate measurement of the ${}^{1}J_{CC}$ coupling constants with an F_1 resolution of < 2 Hz.

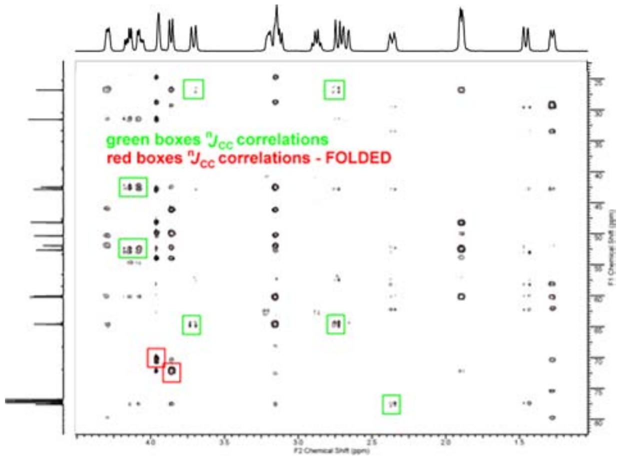

Figure 4. Expansion of the aliphatic region of the ${}^{n}J_{CC}$ *J*-modulated ADEQUATE spectrum acquired using a 4.6 mg sample of strychnine dissolved in 30 μL of deuterochloroform using a Bruker 600 MHz instrument equipped with a 1.7 mm TXI ${}^{1}H/{}^{13}C-{}^{15}N$ cryoprobe. The data were acquired in 60 h as 2048 × 320 points with an F_1 spectral window of 70 ppm with 192 transients accumulated per t_1 increment with the scaling factor set to 20.

The next step of the study was to measure the ${}^{n}J_{CC}$ coupling constants. In an analogous fashion to the ${}^{1}J_{CC}$ coupling constants, these values were measured with a *J*-modulated 1,n-ADEQUATE experiment. These data are shown in Figure 4, and the measured couplings are summarized in Table 1. The parameters used for acquisition of these data were acquired with ${}^{1}J_{CC}$ and ${}^{n}J_{CC}$ values of 50 and 5 Hz, respectively. Accurate determination of the much smaller ${}^{n}J_{CC}$ values required a larger scaling factor (n = 20 or 25) compared to the *J*-modulated 1,1-ADEQUATE experiments. In addition, the number of increments of the evolution period was increased from 200 to 320 and the spectral window was reduced from 180 ppm for the *J*-modulated 1,1-ADEQUATE exeperiment to 70 ppm for the ${}^{n}J$-modulated 1,n-ADEQUATE experiment. These adjustments improved the F_1 digital resolution to < 0.5 Hz.

Theoretical calculations of carbon–carbon spin–spin coupling constants were done based on the density functional theory (DFT) method. Prior to coupling calculations, the molecular geometry of strychnine was fully optimized at the B3LYP/6-31G(d) level of theory. Carbon–carbon spin–spin couplings were then calculated in two steps[15] at the B3LYP/6-311+G(d,p) level. First, Fermi contact (FC) contributions were calculated separately from spin-dipolar (SD), paramagnetic spin-orbital

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Table 1. $^3J_{CC}$ Coupling Constants Measured by Extracting Slices from the $^nJ_{CC}$ J-Modulated ADEQUATE Spectrum Shown in Figure 4

$^{13}C-^{13}C$	$^3J_{CC}$ exptl	$^3J_{CC}$ calcd
20,23	5.1	5.0
15,20[a]	7.0	6.7
11,23	4.5	5.0
16,21	4.3	4.2
8,5	4.1	4.7
12,15	3.1	3.7

[a] The H20−C15 $^3J_{CC}$ coupling arises from a combination of the H20−N19−C16−C15 and H20−C21−C14−C15 pathways.

(PSO), and diamagnetic spin-orbital (DSO) contributions to a modified basis set by uncontracting the original basis set and adding tighter polarization functions for the s and d orbitals. The SD, PSO, and DSO contributions were then calculated with the unmodified basis set. The sum of all four contributions was reported as a final spin−spin coupling. The two-step approach to spin−spin coupling calculations has shown better accuracy in comparison with that of the unmodified basis sets.[15] All calculations were carried out using the Gaussian 09 software package.[16] The excellent agreement between the 27 experimental ($^1J_{CC}$ and $^3J_{CC}$) and DFT calculated carbon−carbon spin−spin couplings is shown in Figure 5.

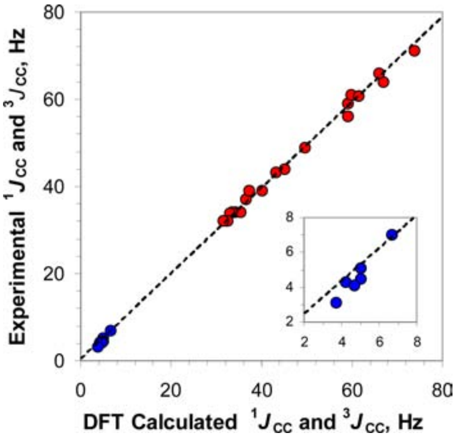

Figure 5. Comparison of the DFT calculated (B3LYP/6-311+G(d,p)) vs measured $^1J_{CC}$ and $^3J_{CC}$ coupling constants for strychnine. Excellent agreement between experimental and calculated values is confirmed by a nearly ideal linear regression described by the 0.98 slope, 0.33 Hz intercept coefficient, 0.9986 correlation coefficient, and 1.09 Hz standard error.

The correlation coefficient r between the two sets of data is 0.9986. The linear regression between experimental and DFT calculated values is described by a nearly ideal 0.98 slope and 0.33 Hz intercept coefficient. A standard error of 1.09 Hz is also comparable to the experimental error. The high accuracy of theoretical calculations combined with the high accuracy of experimental measurements of carbon−carbon couplings (± < 1 Hz), attained by the J-modulated 1,1-ADEQUATE experiment, suggests the considerable predictive power of the method described herein.

Both the $^1J_{CC}$ and $^3J_{CC}$ coupling constants extracted from the J-modulated 1,1- and 1,n-ADEQUATE spectra agreed very well with the values obtained from the DFT calculations. This combination of $^1J_{CC}$ and $^nJ_{CC}$ data together with quantum mechanical calculations should provide investigators with a formidable tool in the study of molecular structure and stereochemical configuration. These experiments and calculations are general in scope and require no prior knowledge of the molecule under study. We have also shown here that these parameters are measurable on reasonable amounts of compounds and in many cases may require less material than standard residual dipolar coupling studies.

Supporting Information Available. Full size expansions of the NMR data shown in Figures 1, 3, and 4; figures showing measurement of $^1J_{CC}$ and $^3J_{CC}$ coupling constants from the F_1 slices; a structure of strychnine illustrating the $^3J_{CC}$ coupling pathways, an enlarged version of Figure 5; and a table of all measured $^1J_{CC}$ and $^3J_{CC}$ coupling constant data for strychnine. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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