Improving NMR methods to solve 3D molecular structure in solution.
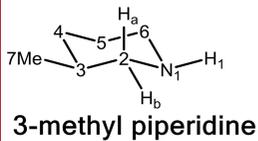
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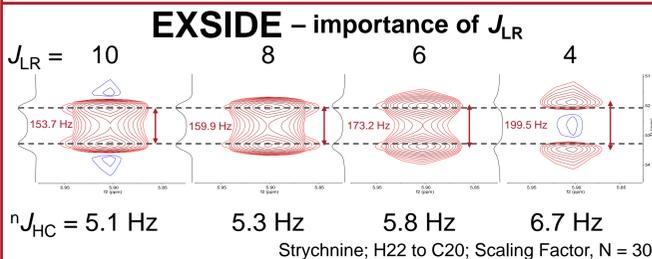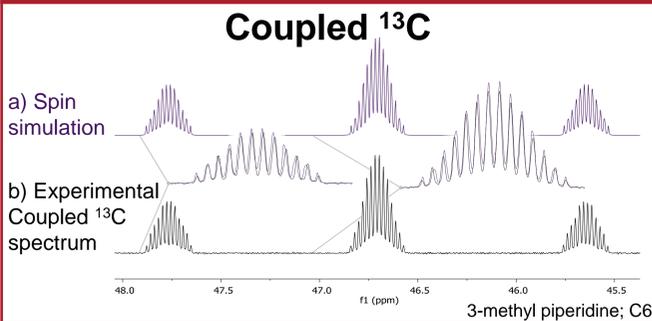
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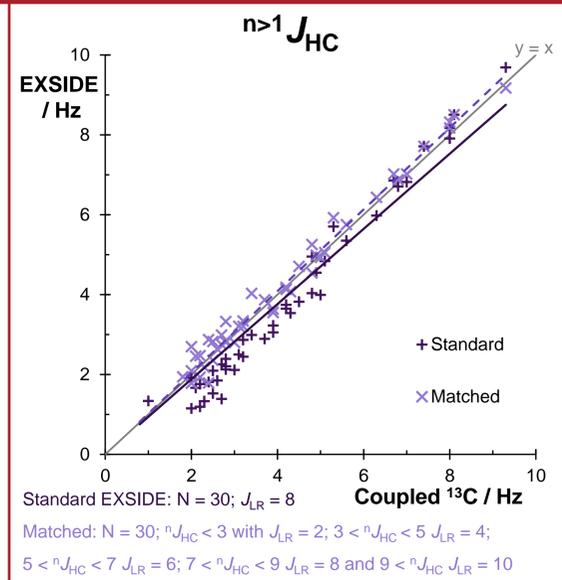
C4X Discovery Better, Safer, Faster

Introduction

Methods of determining scalar coupling constants computationally with Density Functional Theory (DFT) and the performance of empirical equations relating heteronuclear (^{1}H-^{13}C) coupling constants to structure were evaluated. These methods were compared to accurate $^nJ_{HC}$ ($n \geq 1$) experimental values – specifically those derived from coupled ^{13}C spectra – and further compared to a 2D experimental method, EXSIDE [1] to explore the accuracy of the latter. These techniques were applied to strychnine and 3-methyl piperidine and show that there is room for improvement in both experimental and computational approaches to $^nJ_{HC}$ determination.
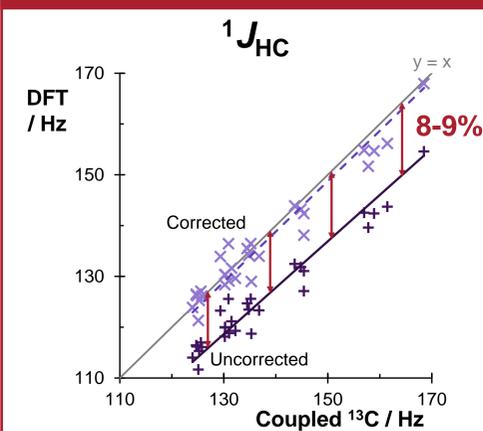
3-methyl piperidine

Strychnine

Experimental measurement of $^nJ_{HC}$

Coupled ^{13}C

a) Spin simulation

b) Experimental Coupled ^{13}C spectrum

3-methyl piperidine; C6

EXSIDE – importance of J_{LR}

J_{LR} = 10   8   6   4

153.7 Hz   159.9 Hz   173.2 Hz   199.5 Hz

$^nJ_{HC}$ = 5.1 Hz   5.3 Hz   5.8 Hz   6.7 Hz

Strychnine; H22 to C20; Scaling Factor, N = 30

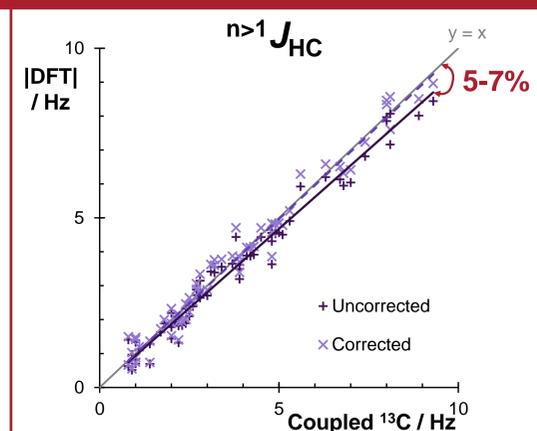○ Coupled ^{13}C spectra were analysed and assigned by manual spin simulation of the multiplets (example upper left).

○ $^{n>1}J_{HC}$ can be measured more readily from simple doublets in 2D EXSIDE data (lower left).

○ EXSIDE showed a 0.5 Hz mean Standard Deviation in $^{n>1}J_{HC}$ measured with $J_{Long Range}$ (J_{LR}) = 2, 4, 6, 8 and 10 Hz.

○ Standard EXSIDE parameters were found to systematically underestimate $^{n>1}J_{HC}$ (right, dark purple, Mean Absolute Error 0.5 Hz and SD 0.4 Hz).

○ Matching of the evolution delays to the magnitude of the expected coupling constant improved accuracy (right, light purple, MAE 0.3 Hz and SD 0.3 Hz).
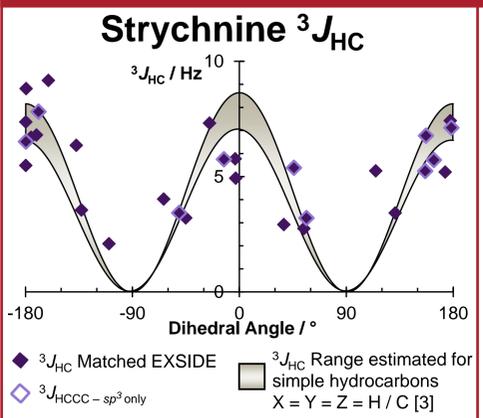
$^{n>1}J_{HC}$

EXSIDE / Hz

+ Standard
× Matched

Coupled ^{13}C / Hz

Standard EXSIDE: N = 30; J_{LR} = 8

Matched: N = 30; $^nJ_{HC}$ < 3 with J_{LR} = 2; 3 < $^nJ_{HC}$ < 5 J_{LR} = 4; 5 < $^nJ_{HC}$ < 7 J_{LR} = 6; 7 < $^nJ_{HC}$ < 9 J_{LR} = 8 and 9 < $^nJ_{HC}$ J_{LR} = 10

Computation of $^nJ_{HC}$

$^1J_{HC}$

DFT / Hz
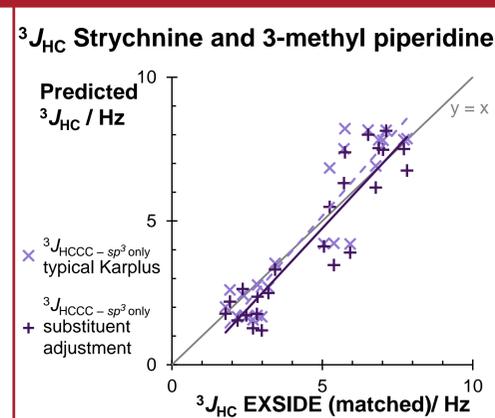
8-9%

Corrected

Uncorrected

Coupled ^{13}C / Hz

○ $^nJ_{HC}$ were calculated using the method described by Bifulco et al [2] (DFT: mPW1PW91/6-311g dp) for strychnine and 3-methyl piperidine (4 conformers).

○ $^1J_{HC}$ showed an 8-9% underestimation of the calculated coupling constants (left, dark purple, MAE 12 Hz, SD 3 Hz). Applying an ~9% correction factor (light purple) reduced the MAE (2 Hz) and SD (2 Hz).

○ Long range $^{n>1}J_{HC}$ found a similar 5-7% underestimation of the coupling constants (right, dark purple, MAE 0.4 Hz and SD 0.3 Hz). Applying a ~6% correction factor (light purple) reduced the MAE (0.3 Hz) and SD (0.2 Hz).

$^{n>1}J_{HC}$

|DFT| / Hz

5-7%

+ Uncorrected
× Corrected

Coupled ^{13}C / Hz

Empirical prediction of $^3J_{HC}$

Strychnine $^3J_{HC}$

$^3J_{HC}$ / Hz

Dihedral Angle / °

◆ $^3J_{HC}$ Matched EXSIDE
◇ $^3J_{HCCC-sp^3}$ only
▨ $^3J_{HC}$ Range estimated for simple hydrocarbons X = Y = Z = H / C [3]

○ Karplus equations relating $^3J_{HC}$ to the dihedral angle between the nuclei were evaluated. The effects of β and γ substituents were accounted for by the equation generated by Bifulco et al [3] (below).

○ Strychnine (left) shows 30 $^3J_{HC}$ measureable by EXSIDE (MAE 1.4 Hz and SD 1.1 Hz), however only 10 do not include sp^2 carbon atoms or heteroatoms in the coupling pathway.

○ $^3J_{HCCC}$ (sp^3 only) for strychnine and 3-methyl piperidine was poorly estimated by only considering the dihedral angle (right, light purple, MAE 0.9 Hz and SD 0.7 Hz).

○ Accounting for substituent effects did not significantly improve the estimation (right, dark purple, MAE 0.8 Hz, SD 0.6 Hz).

$^3J_{HC}$ Strychnine and 3-methyl piperidine

Predicted $^3J_{HC}$ / Hz

× $^3J_{HCCC-sp^3}$ typical Karplus
+ $^3J_{HCCC-sp^3}$ only substituent adjustment

$^3J_{HC}$ EXSIDE (matched)/ Hz

P_n parametrised constants
φ dihedral angle
ξ_i +1 or -1 orientation factor
$\Delta\chi$ electronegativity relative to H

$$^3J_{HC} = P_1\cos^2\varphi + P_2\cos\varphi + P_3 + \sum \Delta\chi_{H\pm}[P_4 + P_5\cos^2(\xi_i\varphi + P_6|\Delta\chi_{H\pm}|)] + \sum \Delta\chi_{C\pm}[P'_4 + P'_5\cos^2(\xi_i\varphi + P'_6|\Delta\chi_{C\pm}|)]$$

Typical form of Karplus Equation   Adjustment for electronegativity and orientation of β and γ substituents [3]

Determining ξ_i
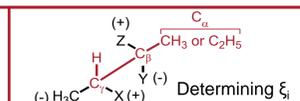
Experimental

EXSIDE spectra were run on a Varian VNMRS 500 MHz Direct Drive Spectrometer with Agilent OneNMR probe at 25°C for 3-methyl piperidine. EXSIDE spectra for strychnine and coupled ^{13}C spectra were run on a Bruker AVANCE III HD 500MHz NMR Spectrometer with 5mm DCH 13C-1H/D Cryo Probe at 25°C. Data were processed and manually fitted spin simulations performed using MestReNova 9.0.1.

The Gaussian 09 software package was used for geometry optimisation and NMR calculations to obtain total scalar coupling constants by the GIAO method using DFT with the mPW1PW91 functional and the 6-311g dp basis set [2] and IEFPCM solvent model for strychnine and 3-methyl piperidine. The four lowest energy conformers of the 3-methyl piperidines were manually generated, single point energies were calculated for each conformer and frequencies were calculated to characterise the minima and provide zero point and entropic corrections to the electronic energy. The Boltzmann distribution was used to estimate populations.

References

[1] Krishnamurthy, V. V. J. Magn. Reson. Ser. A 1996, 121, 33.

[2] Di Micco, S.; Chini, M. G.; Riccio, R.; Bifulco, G. European J. Org. Chem. 2010, 2010 (8), 1411

[3] Palermo, G.; Riccio, R.; Bifulco, G. J. Org. Chem. 2010, 75 (6), 1982.

Conclusions

Careful consideration of the evolution delays is needed for the accurate measurement of $^nJ_{HC}$ by EXSIDE, matching the parameter J_{LR} to the magnitude of the measured coupling.

Calculation of long range $^nJ_{HC}$ for 3-methyl piperidine and $^1J_{HC}$ both in 3-methyl piperidine and strychnine by DFT showed a significant underestimation of the coupling constants.

Heteronuclear (^{1}H-^{13}C) Karplus equations were not robust, requiring the elimination of a number of $^3J_{HC}$ which included sp^2 carbon centres in the coupling pathway or heteroatoms (nitrogen or oxygen).

Although empirical equations can rapidly relate $^3J_{HC}$ to structure, the more in depth DFT calculations estimated $^{n>1}J_{HC}$ with greater accuracy and can utilise all the measurable coupling constants in a molecule to evaluate the structure.

Acknowledgements