Received: 31 January 2015

Revised: 26 April 2015

(wileyonlinelibrary.com) DOI 10.1002/mrc.4264

Conformational analysis: ³J_{HCOC} and ³J_{HCCC} Karplus relationships for methylene ¹H nuclei

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NAMFIS (*N*MR Analysis of *M*olecular *F*lexibility *I*n Solution) was applied to 1-[2-(benzyloxy)phenyl]ethanone using quantitative ¹H-¹H NOE distances and ³J proton-carbon coupling constant (CC) restraints for averaged methylene proton ³J_{HCOC} and ³J_{HCCC} pathways H₂-³J-X imposed by density functional theory-generated Karplus relationships. Comparison of the NOE-only *versus* the NOE + CC conformational selections illustrates that the experimentally measured average ³J coupling constants of methylene protons can be used for solution conformational analysis, potentially valuable in the study of small-molecule drugs and natural products which lack the typically studied H₁-³J-X Karplus relationships. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: NMR; ¹H; ¹³C; conformational analysis; DFT; Karplus

Introduction

The 'Karplus equation',^[1,2] has become one of the most important contributions to NMR spectroscopy^[3] and conformational analysis, describing the relationship between the vicinal ³*J* coupling constant (CC) and the dihedral angle, Θ , between two spin-coupled nuclei.^[4,5] Originally applied to three-bond homonuclear coupling, the relationship has been extended to heteronuclear coupling and numerous examples of both homonuclear and heteronuclear relationship between the CC and the dihedral angle for *I* ≠ 0 nuclei is a key factor in determining molecular shape, fundamental to the understanding of the interactions between small molecules and proteins and the design of new, more selective, more effective, drugs.

In conformational analysis, the application of a Karplus equation is to translate a ³J CC measurement into a dihedral angle relationship between two nuclei in an environment where 'the dihedral angle adopts a prevailing value over time'.^[11] For protons bound to carbon, there are three cases to consider - CH, CH₂ and CH₃. The free rotation of methyl groups means that ³J coupling information between methyl hydrogen atoms and another nucleus provides little conformational information. Methine (CH) protons are the typical subjects,^[12] offering valuable insight into molecular structure. There is, however, the intermediate case of methylene protons, CH₂; while non-equivalent methylene protons with distinctly different chemical shifts and coupling patterns can essentially be treated in the same way as methine protons, provided a definitive assignment can be made, it is the case of seemingly equivalent methylene protons, exhibiting what appears as a singlet signal in the ¹H NMR spectrum, which presents an interesting problem because of the inability to distinguish between the 'magnetically equivalent' protons and their relationship with a third, coupled nucleus (H or X). The magnetic equivalence is, however, only applicable to the ¹²C isotopomer, in which the coupling to all potential coupling partners (or absence thereof) is the same for both protons. In a ¹³C isotopomer, the three-bond coupling between each of the two methylene partners and a third nucleus is different, because the dihedral angles between those methylene protons and the coupled partner are different, by the Karplus equation, arising from the roughly 108° separation of the protons on the sp³-hybridised

carbon atom. In a molecular system where free rotation is impeded, and where an average ${}^{3}J$ CC of methylene protons can be measured, it should be possible to extract environmental information in a manner similar to that applied to a methine proton. The difficulty in extracting the structural information lies in that while in any individual conformer of a particular geometry each of the two methylene protons sits in a unique isotopomeric coupling environment, the spectroscopic measurement can only observe a conformational average.

Computational chemistry software packages like Gaussian^[13,14] possess the ability to calculate CCs given a fixed geometry, usually the result of another calculation. Typically, the calculation of NMR parameters follows a geometry optimisation. This calculation represents only one conformation and while it is hoped that this is the dominant and energetically most favourable structure (a global minimum), experimental evidence in an NMR spectrum often indicates that this is not the case and that there may be a number of conformations present.^[15]

To investigate the possibility of using average ³*J*CCs of methylene protons in conformational analysis, we have combined a computationally derived assessment of the individual ³*J* coupling relationships with experimentally determined average ³*J* values, obtained from an EXSIDE^[16] experiment, and quantitative NOE-derived intramolecular ¹H-¹H distances and used these parameters to select those conformations likely to represent the solution speciation.

Methods

Synthesis (SI)

1-[2-(Benzyloxy)phenyl]ethanone was prepared by condensation of 2-hydroxyacetophenone with benzyl bromide in refluxing acetone in the presence of anhydrous potassium carbonate.^[17–19]

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NMR data (SI)

Experiments were performed on a Bruker Avance-III 500 spectrometer at 30 °C. The sample for the quantitative NOE experiments (10 mg in 600 μ I) was degassed and sealed to remove dissolved oxygen and prevent moisture absorption during the experiments. Quantitative NOESY experiments were performed with mixing times in the 50–300 ms range, and internuclear distances were calculated from cross-peak volumes at a mixing time consistent with linear behaviour,^[20–28] using the isolated-spin-pair-approximation.^[29] A customised hard-X-pulse EXSIDE-type experiment,^[16,30] was used to measure ³J CCs with selective excitation at the required ¹H frequency using a shaped pulse (180° Gaussian Cascade Q3). Shaped pulses were tested for selective excitation with a customised 1-D DPFGSE experiment.^[31]

Conformers (SI)

A conformational search was performed using Schrodinger's MacroModel^[32,33] software producing 1185 structures. To this was added one conformation determined by single crystal X-ray diffraction.^[34]

DFT calculations (SI)

Calculation of CCs was performed using the Gaussian-09W $(C.01)^{[13,14]}$ suite of programs at the MPW1PW91^[35,36] level with the 6–31 + g(d,p) basis set using the 'mixed' method^[37,38] for the relevant nuclei in restricted geometries. A ³*J* versus dihedral angle (Θ) relationship was established using the optimum Karplus-type relationship, the parameters for which were determined using the Origin software package.^[39]

NAMFIS (SI)

*NMR A*nalysis of *M*olecular *F*lexibility *I*n Solution (*NAMFIS*)^[15,40] is a means by which the individual contributing conformations of a small flexible molecule can be extracted from an average NMR spectrum. A set of possible conformations is generated, and these are compared with the experimentally determined proton-proton distances, from quantitative Nuclear Overhauser Enhancement measurements (NOE), and/or dihedral angles, from ³*J* CC measurements. The result is a best-fit set of conformers, characterised by a sum-of-squared-differences (SSD) value, each associated with a mole fraction of the total population.

Results and discussion

While ostensibly simple, 1-[2-(benzyloxy)phenyl]ethanone, *abbr* A2OB, (Figs 1 and 2) presents a number of attractive features:

- The methylene signal in the ¹H NMR spectrum is a singlet, clearly separated from all other signals, facilitating clean selective irradiation for the EXSIDE^[16] experiment;
- The methylene moiety is part of the bridge between two aromatic rings, with ³ J coupling pathways to both rings, potentially offering structural insight into the spacial relationship between the bridge and both rings;
- All ¹H signals can be assigned without ambiguity, leading to unambiguous NOE correlations;
- The single methylene fragment presents only four ³ *J* coupling pathways, computationally manageable at a reasonable level of theory;



Figure 1. Relevant atom numbers from the Gaussian and MacroModel atom number maps, arrowed NOE contacts between ¹H nuclei, and internuclear distances (Å).



Figure 2. HSQCETGPLRJC spectrum of A2OB in DMSO-d₆.

- The aryl-O-CH₂-aryl fragment occurs in 126 small molecule structures found in the Protein Data Bank,^[41] more than 1700 structures in the Cambridge Structural Database,^[42] and forms a component of a novel range of COX-2 inhibitors,^[43]
- The *aceto*-substituent impedes internal movement of the structure and creates the possibility that *the dihedral angle adopts a prevailing value over time*.^[11]

From the NOESY spectrum of A2OB, there are five structurally significant NOE contacts (Fig. 1), together with the reference distance of 2.5 Å.

There are four structurally significant ³*J* coupling relationships for the bridge between the aromatic rings, shown for the relevant isotopomers in Table 1.

Using the HSQCETGPLRJC^[16,30] experiment, the average longrange CCs of the methylene protons have been determined. The spectrum is shown in Fig. 2, providing the average ³J CCs between the methylene protons involved in the three-bond-to-carbon contacts in the aromatic rings, as well as the average ²J CC between

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the methylene protons and the quaternary carbon of the terminal phenyl ring. The spectral region encompassed by the singlet at 5.24 ppm in the ¹H spectrum corresponds to a number of methylene signals, that for the all-¹²C isotopomer, and, masked by this, the methylene proton signals of the various ¹³C isotopomers – these are the species that provide all of the ¹³C information. By irradiating the region of the ¹²C isotopomer, one simultaneously irradiates the signals of the ¹³C isotopomers, even though those signals are masked (excluded from this are the ¹J¹H-¹³C isotopomer signals, visible in the ¹H spectrum as ¹³C satellites).^[44]

From the splitting of the signals in the indirect dimension, the absolute values of the ³J CCs can be determined as ³J_{HCOC} = 2.65 Hz and ³J_{HCCC} = 4.11 Hz.

Using the results of the CC calculations from Gaussian,^[13,14] for 23 restricted, optimised geometries, the ³ *J versus* dihedral angle relationships between the methylene protons (atoms 25 and 26 in Fig. 1) and the appropriate carbon atoms for the ¹³C isotopomers (atoms 2 and 13 in Fig. 1), have been determined (Figs 3 and 3a-SI). The isotopomer with a ¹³C atom located at atom position 14 is the same as that with a ¹³C atom at position 13.

Different forms of the Karplus equation can be found in the literature.^[5,45,46] The curves presented in Figs 3 and 3a-SI have been plotted according to the relationship shown in Eqn (1),^[47] for a phase-shifted form^[48] of the equation, and the values for the constants are presented in Table 2.

³
$$J = \mathbf{A} \cdot \cos^2(\Theta + \mathbf{D}) + \mathbf{B} \cdot \cos(\Theta + \mathbf{D}) + \mathbf{C}$$
 (1)

The plots illustrate that dihedral angles delivering appropriate average ${}^{3}J$ CCs are -92° and -24° (in terms of angle 25-11-10-2) for the average pathway 25/26-10-11-2, and -164° , -77° , $+9^{\circ}$ and



Figure 3. ${}^{3}J_{HCOC}$ for pathways 25/26-10-11-2 and average ${}^{3}J_{HCOC}$, plotted as a function of dihedral angle 25-11-10-2. The offset between 25 and 26 in relation to 2 is 116i° ± 5° over 23 geometries.

Table 2.	Values	of	Karplus	equation	parameters	(A,	Β,	C and	D) for
Equation	(1)								

Pathway/ constant	³ J _{HCOC} (25-11-10-2)	³ J _{HCOC} (26-11-10-2)	³ J _{HCCC} (25-11-12-13) ^a	³ J _{HCCC} (26-11-12-13) ^a			
А	8.4 ± 0.1	8.4 ± 0.1	4.0±0.2	3.7 ± 0.1			
В	-1.7 ± 0.1	-1.6 ± 0.1	-0.9 ± 0.1	-0.8 ± 0.05			
С	-0.1 ± 0.07	-0.1 ± 0.06	1.9 ± 0.1	2.3 ± 0.05			
D	-3.0 ± 0.3	2.8 ± 0.3	14.6 ± 0.7	-12.0 ± 0.9			
^a Similar values obtained for pathways 25-11-12-14, 26-11-12-14; isotopomer with a 13 C atom located at atom position 14 (SI).							

+108° (in terms of 25-11-12-13) for average pathway 25/26-11-12-13. Using the NOE and average ³ *J* CC constraints, with the density functional theory (DFT)-derived Karplus relationships, the *NAMFIS* scripts make a best fit selection of from a set of conformational candidates (SI). The outcome of the process for the NOE-based and NOE + CC-based selections is shown in Table 3; the selected conformers and their relative populations (SI). Common conformers are highlighted in **bold** text (**220**, **711**).

The SSD for each selection is zero and, ironically, disappointing because this provides no immediate guide as to the comparison of the sets. From a relatively large number of conformers, it is easy for *NAMFIS* to provide an excellent match. Even with this wide selection (1185 + 1), in which we could potentially find a completely different conformational mixture for each of the two sets, it is pleasing to note that there is a significant degree of cross-over (33–40%), in the selection of conformers 220 and 711. The X-ray conformation is not selected despite reports of other *NAMFIS* studies indicating the presence of the solid-state structure amongst the solution conformations.^[49–52]

The dihedral angles for the chosen conformers of the NOE-based selection (Fig. 4) are shown in Table 4, with the calculated average ³ J CCs according to the DFT-Karplus equations.

Keeping in mind that the selection in the table is based solely upon six NOE-distances, there is a pattern in the conformers that have been chosen by *NAMFIS* – conformers that present ${}^{3}J_{\rm HCOC}$ values of around 6 Hz, 85% of the population, despite a variety of 25-11-10-2 angles. Those conformers with 25-11-10-2 angles in the range $-161^{\circ}\pm12^{\circ}$ represent 60% of the population. The obvious 'odd man out' is 711 (14%), with a 25-11-10-2 angle of -68° and a ${}^{3}J_{\rm HCOC}$ value of 1 Hz, the closest (albeit poor) match to the experimentally measured average ${}^{3}J_{\rm HCOC}$ CC (2.6 Hz).

There is little common ground amongst the 25-11-12-13 dihedral angles although there is a strong presence of conformers presenting ${}^{3}J_{HCCC}$ values around 4 Hz (220, 1020, 763, 746 and 760), some 60% of the mixture, with selections presenting ${}^{3}J_{HCCC}$ values around 5 Hz (711, 1121, 748, 1137 and 1146) making up the remainder of the mixture. Conformer 763 (13%) represents the closest individual match to the experimentally measured average ${}^{3}J_{HCCC}$ CC (4.3 vs 4.1 Hz).

While the focus of the analysis is the link between the two rings, the relative position of the acetyl group cannot be ignored. Governed by three distance constraints (Fig. 1), most of the selected conformers have the methyl hydrogen atoms turned inward (70%), towards the ether moiety (Fig. 4a-SI). The obvious misfits, based on a graphical inspection, are conformers 711, 746, 760 and 1146 (30%) with the acetyl group turned carbonyl-inwards or side-on to the benzyl ether (Fig. 4b-SI).

The dihedral angles for the chosen conformers of the NOE + CCbased selection (Fig. 5) are shown in Table 5, with the calculated average ³ J CCs according to the DFT-Karplus equations.

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Table 3. NMR Analysis of Molecular Flexibility in Solution conformational selections

	A2OB (NOE)	A2OB (NOE + CC) [¹³ C iso-13] ^a	A2OB (NOE + CC) [¹³ C iso-14] ^a
Number of candidate conformers	1185 + 1 (XRD)	1185 + 1 (XRD)	1185 + 1 (XRD)
Number of NOE, J constraints	6	6, 2	6, 2
Number of selected conformers	10	8	8
Conformer, (%)	220 (18.6)	711 (31.9)	711 (33.9)
	1020 (16.1)	499 (20.2)	499 (19.7)
	711 (14.5)	1156 (18.0)	1156 (19.2)
	1121 (13.6)	347 (11.7)	1159 (9.0)
	763 (13.5)	220 (8.0)	347 (8.6)
	746 (9.6)	1159 (7.3)	220 (7.0)
	748 (4.7)	1117 (1.5)	1117 (1.5)
	1137 (3.7)	1120 (1.4)	1120 (1.3)
	1146 (3.4)	_	
	760 (2.0)	_	_
SSD	0	0	0

CC, coupling constant; XRD, X-ray diffraction; SSD, sum of squared differences; NAMFIS, NMR Analysis of Molecular Flexibility in Solution.

^a*NAMFIS* analysis of the isopomerically identical ¹³C-at-position-13 and ¹³C-at-position-14 delivers the same selection of conformations, in the same order, up to the 70% level; thereafter, the conformers selected are the same with small differences in the population levels.



Figure 4. NMR Analysis of Molecular Flexibility in Solution conformational selection (NOE-based), ten conformers.



Figure 5. NMR Analysis of Molecular Flexibility in Solution conformational selection (NOE + coupling constant-based), eight conformers.

Table 4. NMR Analysis of Molecular Flexibility in Solution conformational selection, NOE-based (SI)							
Conformer (%)	Angle 25–2 (°)	Angle 26–2 (°)	³ J _{HCOC} (Hz)	Angle 25–13 (°)	Angle 26–13 (°)	³ J _{HCCC} (Hz)	
220 (18.6)	-166.1	-51.3	6.1	-72.0	+170.6	4.4	
1020 (16.1)	-151.9	-36.3	6.4	-86.6	+157.8	3.7	
711 (14.5)	-68.1	+52.0	1.0	-13.8	-133.2	5.2	
1121 (13.6)	+40.2	+156.4	6.4	+176.9	+60.7	5.0	
763 (13.5)	-150.9	-36.5	6.3	+109.8	-5.4	4.3	
746 (9.6)	+43.7	+158.9	6.3	-156.9	+87.9	3.7	
748 (4.7)	-173.1	-59.5	5.7	+128.6	+6.8	5.2	
1137 (3.7)	-161.7	-45.4	6.3	-56.8	-173.0	5.1	
1146 (3.4)	-163.4	-46.3	6.3	-52.0	-168.7	5.2	
760 (2.0)	+39.6	+154.6	6.3	+13.7	-101.5	3.7	

Applying the NOE-distance and the CC constraints, *NAMFIS* selects a somewhat different set of conformations. Conformers 711, 347 and 1159 have 25-11-10-2 angles in the region $-61^{\circ} \pm 7^{\circ}$ and ${}^{3}J_{\text{HCOC}}$ values of around 1 Hz (50% of the population). Excluding

the conformers at < 2% level, the remainder of the selection is made up of three conformers, 499, 1156 and 220. These three conformers have completely different 25-11-10-2 angles but conformers 1156 and 220 (26% of the population) present ${}^{3}J_{\rm HCOC}$

Table 5. NMR Analysis of Molecular Flexibility in Solution conformational selection, NOE + coupling constant-based								
Conformer (%)	Angle 25–2 (°)	Angle 26–2 (°)	³ J _{HCOC} (Hz)	Angle 25–13 (°)	Angle 26–13 (°)	³ J _{HCCC} (Hz)		
³ J constraint	_	_	2.6	_	_	4.1		
711 (31.9)	-68.1	+52.0	1.0	-13.8	-133.2	5.2		
499 (20.2)	-89.4	+28.4	2.3	-85.2	+158.7	3.8		
1156 (18.0)	+37.2	+152.3	6.3	-139.1	+105.4	3.0		
347 (11.7)	-54.6	+63.2	1.0	+73.3	-42.9	2.5		
220 (8.0)	-166.1	-51.3	6.1	-72.0	+170.6	4.4		
1159 (7.3)	-58.9	+59.6	1.0	+151.3	+34.2	5.6		
1117 (1.5)	-82.5	+32.7	2.0	-109.7	+135.0	2.9		
1120 (1.4)	-75.6	+43.1	1.4	-98.9	+144.8	3.2		

Table 6. Dihedral angles and density functional theory-calculated average ³ J coupling constants for conformers X-ray diffraction, 499, 711 and 821								
Conformer	Angle 25–2 (°)	Angle 26–2 (°)	³ J _{HCOC} (Hz)	Angle 25–13 (°)	Angle 26–13 (°)	³ J _{HCCC} (Hz)		
³ J constraints	_	_	2.6	_	_	4.1		
XRD	-58.4	+61.2	0.9	-33.3	-152.9	5.6		
499	-89.4	+28.4	2.3	-85.2	+158.7	3.8		
711	-68.1	+52.0	1.0	-13.8	-133.2	5.2		
821	-59.5	+59.5	0.9	+149.0	+31.8	5.6		
XRD, X-ray diffraction.								



Figure 6. NMR Analysis of Molecular Flexibility in Solution conformers 499 (light), 711 (medium) and X-ray diffraction (dark).

values of around 6 Hz, consistent with the majority of the NOEbased selection. Conformer 499 (20%), with a 25-11-10-2 angle of -89° , presents the closest ${}^{3}J_{\text{HCOC}}$ value (2.3 Hz) to the constraint (2.6 Hz).

There is a less commonality amongst the NOE + CC conformers in terms of the 25-11-12-13 angle and the ${}^{3}J_{HCCC}$ values. Notably, the 'odd man out' from the NOE selection, conformer 711, is the top selection, fitting neatly into the NOE + CC-based selection, with ${}^{3}J_{HCCC}$ of 1.0 Hz and ${}^{3}J_{HCCC}$ of 5.2 Hz, at a substantial level of 32%. Conformer 499 (20%) presents the closest match to the ${}^{3}J_{HCCC}$ constraint, followed closely by conformer 220 (8%), common to both selections.

With regard to the acetyl substituent, conformers with the methyl hydrogen atoms turned inward (Fig. 5a-SI) dominate the selection (98%). The obvious misfit (Fig. 5b-SI), with the acetyl group turned carbonyl-inwards, is conformer 1117 (2%).

Consideration has been given to the HCOC and HCCC bond angles for pathways 25/26-11-10-2 and 25/26-11-12-13, and the resultant average ³*J* CCs that arise from the conformation of A2OB as determined by X-ray diffraction,^[34] based on the proposal that this conformation may be present in solution,^[49–52] although this is not always the case.^[47] There is an excellent match for the crystal structure in the conformer pool, conformer 821 (Fig. 5c-SI), but the crystal structure (XRD) has been added anyway.

The values for the angles of the pathways and the calculated ${}^{3}J$ CCs for conformer XRD are presented in Table 6 – dihedral angles for pathways 25/26-11-10-2 of -60° and $+60^{\circ}$, respectively, with a ${}^{3}J_{\rm HCOC}$ value of 0.9 Hz, dihedral angles for pathways 25/26-11-10-13 of -30° and -150° , respectively, and a ${}^{3}J_{\rm HCCC}$ value of 5.6 Hz. Amongst the common conformers, conformer 711 provides the closest match within the NOE-based (14%) and the NOE+CC-based (32%) selections.

The best individual match for the CC constraints is conformer 499, with ${}^{3}J_{HCOC}$ of 2.3 Hz and ${}^{3}J_{HCCC}$ of 3.8 Hz and angles 25-11-10-2 of -89° and 25-11-12-13 of -85° (Table 6). While conformer XRD is not chosen by *NAMFIS*, the root mean square deviation on an 'all atoms' basis between this conformation and conformer 499 is 0.9, and for conformer 711, $1.0^{[53]}$ (Fig. 6).

Despite the similar root mean square deviation values, it is clear from the graphical comparison (Fig. 6a-SI) that conformer 499, from the application of CC constraints, is a better match for the crystal structure.

Conclusion

NAMFIS has been applied to a key conformational element of 1-[2-(benzyloxy)phenyl]ethanone using ¹H-¹H distances and ¹H-¹H distances combined with DFT-calculated average coupling constants for H₂-³ J-X pathways. Good agreement between the distance-only and the distance-CC conformational selections indicates that the theoretical treatment of average ³ *J* versus Θ relationships for methylene protons, combined with measured average ³ *J* coupling constants, is a potentially useful means of providing structural insight in cases of restricted movement of methylene groups.

Acknowledgements

The authors acknowledge the support of the University of KwaZulu-Natal (Pietermaritzburg) and the National Research Foundation (NRF) of South Africa.

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