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More accurate $^1J_{CH}$ coupling measurement in the presence of $^3J_{HH}$ strong coupling in natural abundance

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ABSTRACT

J couplings are essential for measuring RDCs (residual dipolar couplings), now routinely used to deduce molecular structure and dynamics of glycans and proteins. Accurate measurement of $^1J_{CH}$ is critical for RDCs to reflect the true structure and dynamics in the molecule of interest. We report noticeable discrepancies between $^1J_{CH}$ values measured with HSQC type pulse sequences in the ^{1}H dimension from those measured in the ^{13}C dimension for 17 sugars and show that these discrepancies arise from strong scalar coupling. In order to determine how to minimize errors in measuring $^1J_{CH}$, we analyze the strong coupling effects in detail using the product operator-formalism and spectral simulations based on the solution of the Liouville equation (not considering relaxation effects) in the presence of strong coupling. We report that the apparent $^1J_{CH}$ measured with 2D HSQC-based sequences in either dimension can be in error by up to 4 Hz and that the values measured in the ^{1}H dimension can disagree with those in the ^{13}C dimension by up to 7 Hz. We demonstrate that spectral simulations can reproduce the errors induced by strong coupling and that these can be used to extract true $^1J_{CH}$ values. We find that the $^1J_{CH}$ values measured using a modified Z-filtered coupled HSQC are still affected by strong coupling. We conclude that spectral simulation yields accurate $^1J_{CH}$ with errors as low as 1% in the presence of strong coupling.

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1. Introduction

^1J (one-bond *J* couplings) have found application in RDC (residual dipolar couplings) measurements because they can aid in structural analysis by relating the orientations of two non-interacting bonds [1,2]. RDCs are also used to probe dynamics of molecules [3–5]. Usually RDCs are calculated from the difference between ^1J measured in isotropic and weakly aligned media, (^1J + RDC). Consequently, accurate ^1J are paramount for obtaining reliable structures and dynamics using RDCs. Since the introduction of RDCs, methods including frequency difference and quantitative *J* have been used to measure ^1J [6–10]. Frequency difference methods are the most widely used since they are simple and efficient. Our focus in this paper is to examine the accuracy of $^1J_{CH}$ measured by coupled HSQC experiments. Frequency differences between doublet components are commonly measured in either the direct or indirect dimension of a heteronuclear correlation spectrum. However, the accuracy of $^1J_{CH}$ has not been evaluated, especially in a strongly coupled system, typically found in carbohydrates. In using these methods it is usually assumed that the NMR spectra are first order and that the frequency differences between the doublet components are the true $^1J_{CH}$ values, while effects from strong coupling (second-order effects) are ignored [7,8].

In this report we show that there are discrepancies between $^1J_{CH}$ values measured in the ^{1}H dimension and their corresponding values measured in the ^{13}C dimension of two-dimensional NMR correlation spectra. Using the product-operator formalism [11] and spectral simulations, we find that many measurements are in error in both dimensions and that the discrepancies are caused by strong ^{1}H–^{1}H coupling, which results in a change in both the resonance frequency and relative intensities of doublets components, and the presence of spurious peaks.

Importantly, these errors do not cancel when comparing data from isotropic and anisotropic media. We demonstrate that more accurate $^1J_{CH}$ values can be obtained from spectral simulations by taking strong coupling into account. Finally, we evaluate the accuracy of $^1J_{CH}$ from a modified Z-filtered HSQC.

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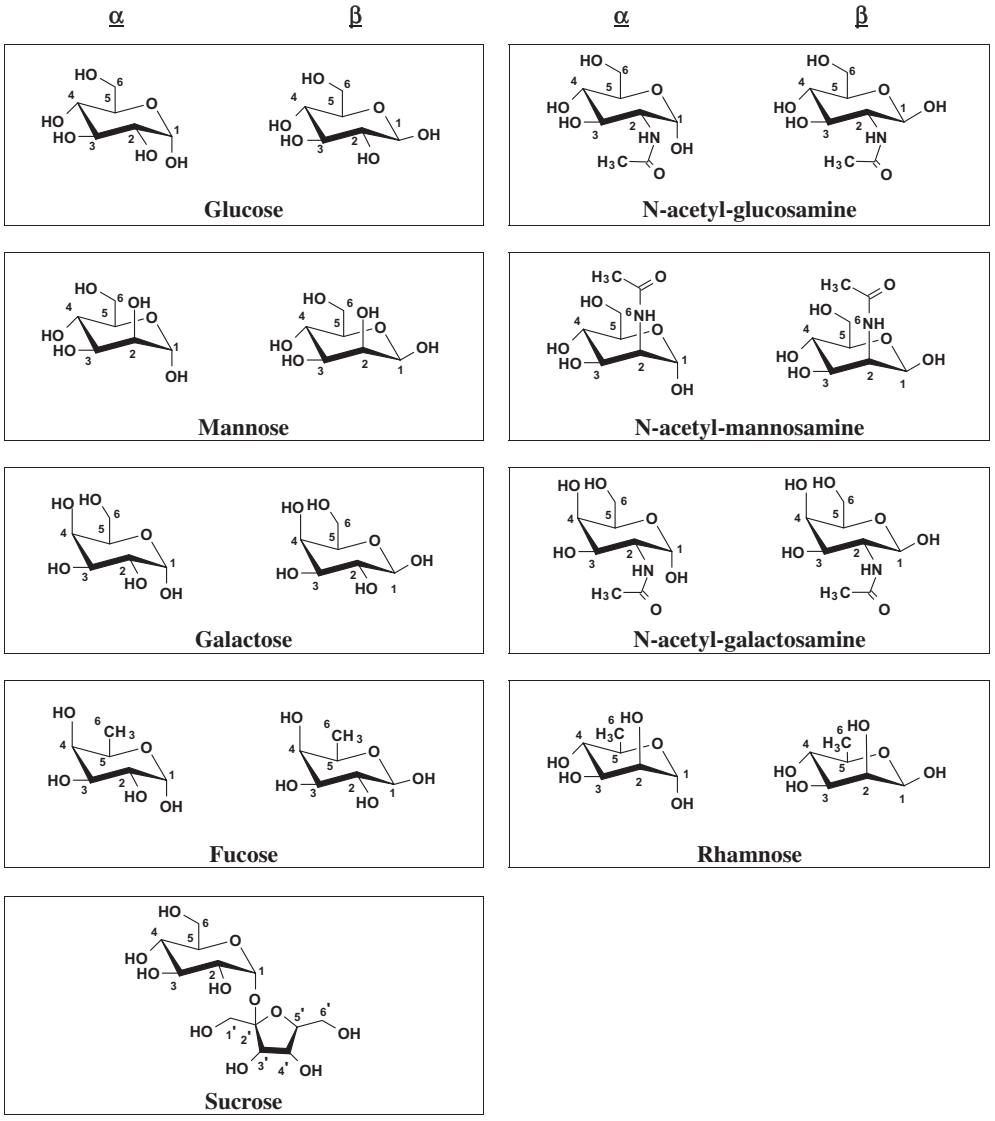

Fig. 1. Structures of 17 sugars used for $^1J_{CH}$ measurement. Each monosaccharide sample is an equilibrium mixture of α and β anomers in slow exchange at 37 °C, generating anomer specific NMR spectra.

2. Materials and methods

2.1. NMR spectroscopy

Non-labeled glucose, galactose, mannose, rhamnose, fucose, *N*-acetyl-glucosamine, *N*-acetyl-galactosamine, *N*-acetyl-mannosamine (Fig. 1) and D_2O (99.9%) were purchased from Sigma–Aldrich (St. Louis, USA). Sucrose was purchased from Domino Foods (Iselin, NJ). The sugars were used without further purification. Each sugar (5 mg) was dissolved in 500 μL of D_2O in a 5 mm Wilmad NMR tube (Buena, NJ). All experiments were performed at 37 °C on an AV-II-700 Bruker-Biospin NMR spectrometer (Billerica, MA). For all 2D heteronuclear correlation experiments, the ^{1}H carrier was set at 4.72 ppm and the ^{13}C carrier was set at 69 ppm. Spectra were acquired with spectral windows of 4.2 ppm and 20 ppm for ^{1}H and ^{13}C, respectively; a recycle delay of 1 s and 16 scans per t_1 point. The INEPT delay τ, $1/(4 * {}^1J_{CH})$ was set to 1.67 ms for a 150 Hz $^1J_{CH}$. 1024 complex points were collected in the ^{1}H dimension and 512 complex points in the ^{13}C dimension to yield a raw digital resolution of 3.8 Hz/point and 9.78 Hz/point in the ^{1}H and ^{13}C dimension, respectively. All spectra were zero-filled to 4096 points in ^{1}H and 2048 points in ^{13}C, unless otherwise stated.

Three closely related types of HSQC experiment were used (Fig. 2). To measure $^1J_{CH}$ in the ^{13}C dimension, the ^{1}H 180° decoupling pulse during the ^{13}C evolution time was removed (Fig. 2a). To measure $^1J_{CH}$ in the ^{1}H dimension, ^{13}C decoupling was turned off during acquisition (Fig. 2b). To remove zero-quantum coherence induced antiphase elements in the ^{1}H dimension, the pulse sequence in Fig. 2b was modified with a Z-filter (Fig. 2c). The Z-filter includes a swept-frequency pulse (1 ms) inserted between the ^{1}H and ^{13}C 90° pulses in the reverse INEPT transfer simultaneously with a 1 ms, 4 G/cm gradient pulse applied to attenuate zero quantum coherence [12]. To remove errors due to pulse imperfections, a 90° ^{13}C purge pulse and a gradient crusher pulse were added at the beginning of the pulse sequence and between the ^{1}H and ^{13}C 90° pulses in the forward INEPT period, respectively in all three pulse-sequences. Each experiment took about 3 h to complete and was repeated four times to evaluate the experimental reproducibility. The raw data were processed with NMRPipe [13].

2D ^{1}H–^{1}H COSY spectra were collected using the standard COSYPH pulse program from the Bruker pulse library, with 2048 points in the direct dimension and 512 points in the indirect dimension. The ^{1}H carrier was placed at 4.72 ppm with spectral windows of 4.2 ppm in both dimensions. The recycle delay was set to 3.5 s.