Accurate Measurement of Vicinal Carbon–Hydrogen Coupling Constants via Ammonium Nitrogen Based on HMBC Experiments

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Vicinal spin–spin coupling constants frequently used for conformation analysis were determined for ammonium-containing ^{13}C–N–C–^{1}H systems by using HMBC. 1-Deoxynojirimycin hydrochloride provided an appropriate system for measuring the antiperiplanar and gauche interactions, which were determined to be 7.3 and 1.6 Hz, respectively.

Spin–spin coupling constants are frequently used for the conformational analysis of bioorganic molecules including proteins and polysaccharides.[1–3] Besides proton–proton interaction such as $^{3}J_{HH}$, vicinal carbon–proton constants ($^{3}J_{CH}$) are often utilized in various fields of organic and bioorganic chemistry.[4,5] For example, "*J*-based configuration analysis (JBCA)"[6] has been developed for the determination of relative stereochemistry in acyclic systems of natural and synthetic compounds chiefly based on $^{2,3}J_{CH}$ and $^{3}J_{HH}$. Recently a number of applications of this method have been reported,[7–9] thereby being regarded as a standard methodology in natural product chemistry. However, it is sometimes difficult to directly measure the long-range heteronuclear coupling constants for ^{13}C (1.1%) and ^{15}N (0.37%) in natural abundance.[10–12] The heteronuclear multiple bond correlation (HMBC)[13] technique in combination with a pulse fielded gradient (PFG)[14–18] have facilitated the highly sensitive detection of the long-range coupling correlations for insensitive nuclei.

Amine and ammonium often play a key role in exerting biological activities of natural products and other biomolecules such as hormones and neurotransmitters. Upon examination of their conformation with respect to a C–N bond, vicinal *J* values of ^{13}C–N–C–^{1}H systems provide essential information. However, no reliable $^{3}J_{CNCH}$ data have been obtained except for those of uridine derivatives where the *J* values were determined for sp^2 carbon and hydrogen atoms via nonionizable nitrogen functionality.[19] Biogenic amines are largely ionized in physiological conditions. Moreover, the ionization states and the s-character of carbon atoms greatly influence the size of the *J* values. Thus, for the conformation studies of nitrogen-containing biomolecules such as polyamines, we attempted to determine the accurate $^{3}J_{CNCH}$ for sp^3 carbon atoms via an ammonium group. In this study we report the vicinal coupling constants of ^{13}C–N–C–^{1}H systems containing an ammonium nitrogen atom with antiperiplanar (near 180°) and gauche (near 60°) orientations on the basis of the HMBC spectra of 1-deoxynojirimycin hydrochloride (**1**).

It is considered that **1** adopts a chair conformation which is rigid enough for determining the typical $^{3}J_{C,H}$ values for the antiperiplanar and gauche orientations in aqueous solution; Conformation research of **1** by MacroModel[20] gave rise to a single chair conformer, where the dihedral angles of C5/H-1_{eq} and C5/H-1_{ax} were calculated to be 178.9 and 64.9°, respectively. The overlapping ^{1}H NMR signals of H-1_{eq} and H-3 in D_2O solution[21] were separated by addition of 5% pyridine-d_5, which allowed us to measure $^{3}J_{CNCH,anti}$. Thus, the further NMR experiments were carried out with this solution.[22] The spin–spin coupling constant between H-1_{ax} and H-2 was determined to be 11.5 Hz from a conventional ^{1}H NMR spectrum, indicating the axial orientation of H-2 as well as the chair conformation of **1** (Figure 1). Therefore, the dihedral angles of C5/H-1_{eq} and C5/H-1_{ax} can be regarded as antiperiplanar and gauche orientations, respectively.

We determined the $^{3}J_{CNCH}$ values, using the pulse sequence of a standard HMBC-PFG method[23] with a low-pass *J* filter[24] (Figure 2), in which the cross-peak signal intensity is proportional to $\cos(\pi J_{CH}\Delta_1)\sin(\pi J_{CH}(\Delta_2 - \Delta_1))$.[25] The first 90° ($^{13}C$) pulse after Δ_1 serves as the *J* filter, in which Δ_1 is usually set to $1/2^{1}J_{CH}$ (3–4 ms) for suppressing cross peaks due to one-bond correlations. The duration of Δ_2 depends on the minimum *J*

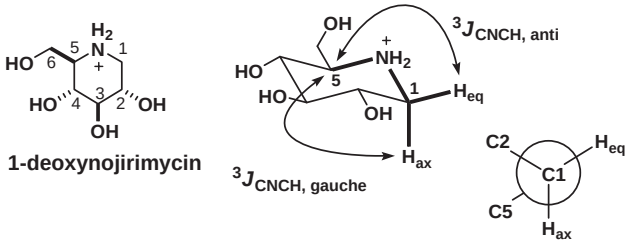

Figure 1. Structure of 1-deoxynoirimycin in ammonium form.

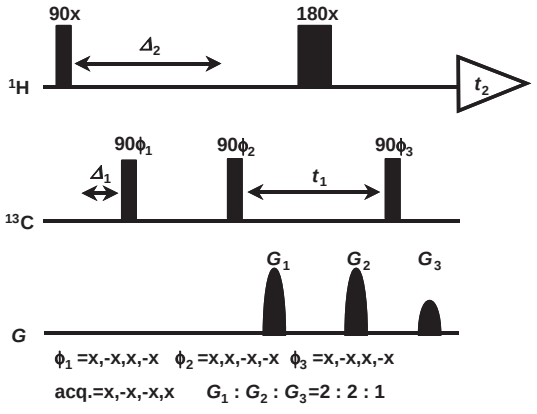

Figure 2. HMBC-PFG pulse-sequence. Δ_1 was changed from 0 to 400 ms in 30 ms steps, during which Δ_2 was kept at 400 ms. t_1 is evolution time. G_1, G_2, and G_3 stand for pulse field gradient (PFG). ϕ_1, ϕ_2, ϕ_3, and *acq.* are phase parameters for ^{13}C 90° pulse and acquisition, respectively.

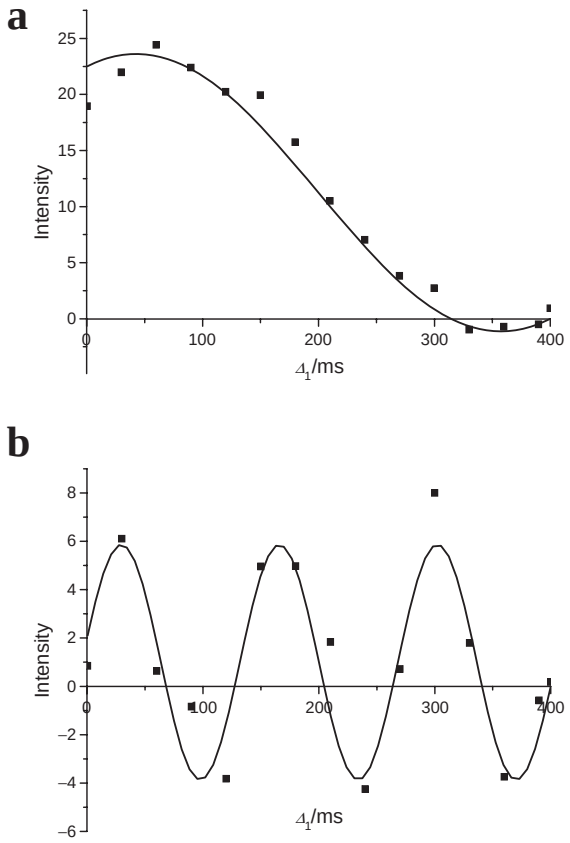

Figure 3. Evolution time-dependent changes of $^3J_{C5/H1ax}$ (**a**) and $^3J_{C5/H1eq}$ (**b**). Squares denote experimental data of the cross peak intensity of HMBC and solid lines are theoretical curves for 1.59 Hz (**a**) and 7.34 (**b**) Hz.

value to be determined (usually set around 60 ms). In this experiment, we measured the signal intensities of **1** by varying Δ_1 from 0 to 400 ms in ca. 30 ms steps, while keeping Δ_2 at 400 ms throughout the experiments. Under these conditions, the intensities of relevant cross peaks follow the correlation shown in Figure 3.

The observed intensities of C5/H1$_{ax}$ were best fitted to theoretical curve of 1.59 Hz (Figure 3a) and those of C5/H1$_{eq}$ agreed well with that of 7.34 Hz (Figure 3b).[26] Consequently, $^3J_{CNCH,anti}$ and $^3J_{CNCH,gauche}$ were determined to be 7.34 and 1.59 Hz, respectively. These $^3J_{CNCH}$ values can be fitted to the Karplus-type relationship reported by Lemieux et al.[19] Further experiments on cyclic amines with different dihedral angles will be necessary for deriving the general Karplus equation for these nitrogen containing systems.

References and Notes

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20 The conformation research of **1** in D_2O was carried out by using an OPLS2001 force field on MacroModel.
21 In the D_2O solution of **1** without C_5D_5N, $^3J_{C5/H1ax}$ and $^3J_{C5/H1eq}$ were determined as 1.5 and 7.3 Hz although their accuracy was not very high owing to the overlapping signals. The effect of pyridine on the size of the *J* values was thus negligible under the experimental conditions.
22 1-Deoxynojirimycin hydrochloride (10 mg, Wako Pure Chemicals) was dissolved in 200 μL of 5% C_5D_5N-containing D_2O. ^{1}H NMR (500 MHz, D_2O–C_5D_5N 19:1): δ 3.77 (H-6), 3.67 (H-6), 3.58 (H-2), 3.38 (H-4), 3.33 (H-3), 3.26 (H-1$_{eq}$), 2.90 (H-5), 2.70 (H-1$_{ax}$).
23 HMBC was performed at 25 °C in a SHIGEMI sample tube on a JEOL ECA-500 (500 MHz) spectrometer. The FID data were acquired with 16 scans per increment for 4096 (F2) × 64 (F1) matrix. The 2D data were processed with Gauss and Sinbell window functions after 4-time zero-filling for the F1 axis.
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25 The observable term selected by PFG passes through single-quantum coherence during Δ_2, and thus undergoes this $J_{C,H}$ evolution.
26 The curve fitting was carried out by software Origin 6.1, which also provided standard errors; 1.591 ± 0.072 and 7.335 ± 0.065 Hz.

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