Rapid Communications

Long-Range $^1H-^{15}N$ Heteronuclear Shift Correlation at Natural Abundance Using Gradient-Enhanced HMQC

Gary E. Martin* and Ronald C. Crouch

Bioanalytical Sciences NMR Laboratory, Burroughs Wellcome Co., Research Triangle Park, North Carolina 27709

Maged H. M. Sharaf and Paul L. Schiff, Jr.

Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

Received September 19, 1995®

Abstract: Long-range $^1H-^{15}N$ GHMQC spectra afford a viable means of beginning to incorporate ^{15}N into strategies to elucidate the structures of potentially novel alkaloids. Long-range $^1H-^{15}N$ heteronuclear shift correlation using a variant of the gradient-enhanced HMQC pulse sequence is demonstrated at ^{15}N natural abundance using the indoloquinoline alkaloid cryptolepine.

Gradient-enhanced inverse-detected heteronuclear shift correlation experiments have several noteworthy attributes. Pulsed field gradients (PFG) can be used to select coherence pathways, thereby eliminating phase cycling schemes and allowing experiments to be performed in far fewer transients/t_1 increment than is possible with conventional pulse sequences. Gradients can also be employed to suppress unwanted resonances, e.g., water. For natural products chemists, gradient-enhanced experiments also have the particularly beneficial effect of eliminating t_1 noise, making it far easier to observe weak (e.g., $^4J_{CH}$) couplings in GHMBC experiments. Unfortunately, gradient-enhanced NMR experiments also have an inherent drawback—the use of gradients leads to ~50% loss of signal. Signal losses aside, gradient-enhanced $^1H-^{15}N$ long-range correlation experiments ($^1H-^{15}N$ GHMQC) are vastly superior to any nongradient $^1H-^{15}N$ correlation experiment of which we are aware. The improvement derives principally through the elimination of t_1 noise afforded by the gradients.

Our initial efforts to employ $^1H-^{15}N$ long-range couplings in alkaloids utilized quindoline, which has an NH in its structure, and cryptolepine (**1**) as model compounds.[1] This work did not employ gradients. In a nongradient experiment with a 10 mg sample of quindoline, the N10–H resonance at 108.6 ppm was readily observed. A 10 Hz long-range optimized experiment contained evidence of a long-range coupling from H4 at 283 ppm in F_1 which was a plausible chemical shift for the quinoline N5 resonance of quindoline. No long-range couplings were observed from either H9 or H11 to the N10 indole resonance in the long-range optimized experiment. Similar results were obtained with cryptolepine. Using nongradient techniques, the coupling between the *N*-methyl singlet and N5 was readily observable. A much weaker coupling was also observed between the H4 doublet and the N5 resonance, consistent with the couplings observed for quindoline. Again, however, no evidence of any coupling from either H9 or H11 to the N10 indole resonance was observed. For this reason, we elected to reexamine the long-range $^1H-^{15}N$ coupling pathways of cryptolepine using gradient-enhanced NMR methods.

We recently reported the first application of gradient-enhanced long-range $^1H-^{15}N$ (GHMQC) heteronuclear shift correlation to ajmaline[2] and several *Strychnos* alkaloids,[3] demonstrating the viability of the method. Using a sample prepared by dissolving 6.5 mg (~20

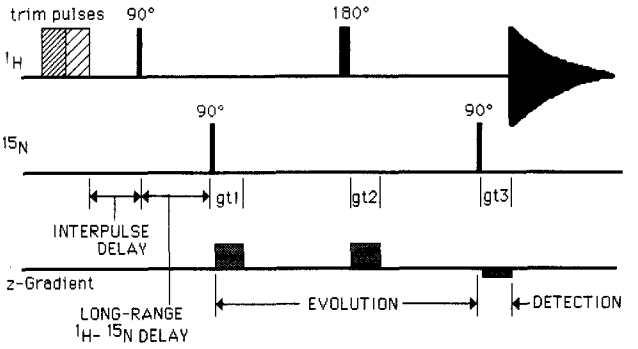
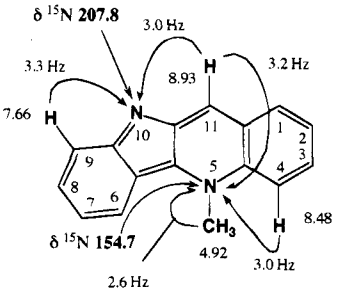


Figure 1. Gradient-enhanced HMQC-derived sequence used for long-range $^1H-^{15}N$ heteronuclear shift correlation. Pulsed field gradients, gt1–gt3, had uniform rise times of 100 μs and durations of 1.5 ms; gradient pairs of 5:5:1 and 5:5:−1 were used with tweaked DAC values of 10 000, 10 000, 2010. This approach gave fully phase-sensitive data, although the absence of 180° ^{15}N refocusing pulses led to a linear phase shift across F_1, thereby allowing only data for one ^{15}N resonance to be correctly phased in F_1 at any given time. ^{15}N refocusing pulses on were avoided in the present work to minimize any potential signal losses which might arise through miscalibration of the 180° ^{15}N pulses. For the same reason, we preferred to employ an HMQC-base pulse sequence rather than one based on an HSQC sequence. The long-range delay for the experimental data shown in Figures 2 and 3 was optimized for 4 Hz (see text). Data were processed using coefficients of (1,0,0,1,0,1,1,0) with the Varian wft2d processing command. The $^1H-^{15}N$ data could also be acquired using gradients of 5:5:1 (processed with coefficients of (1,0,0,1)) or 5:5:−1 (processed with coefficients of (1,0,0,−1)).



1

* Present address: Pharmacia & Upjohn, Inc., Kalamazoo, MI 49001.

® Abstract published in *Advance ACS Abstracts,* December 15, 1995.

0163-3864/96/3259-0002$12.00/0

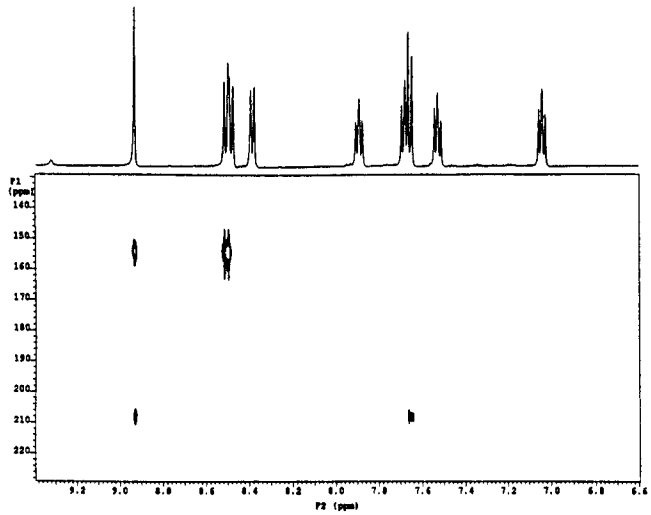
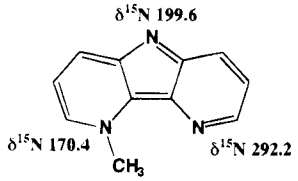


Figure 2. Long-range $^1H-^{15}N$ GHMQC spectrum of a sample of 20 μmol of cryptolepine (**1**) dissolved in 650 μL of DMSO-d_6 showing correlations from H4, H11, and the NMe resonance (latter not shown) to N5 resonating at 154.7 ppm and correlations from H9 and H11 to the N10 indole resonance at 208.7 ppm. The data are presented as a power spectrum in both F_1 and F_2.

μmol) of cryptolepine (**1**) in 650 μL of DMSO-d_6 in a 5 mm tube, a 4 Hz long-range $^1H-^{15}N$ GHMQC was acquired using the pulse sequence shown in Figure 1 on a Varian three-channel Unity 500 spectrometer equipped with a Nalorac Z-SPEC IDTG-500-5 gradient probe. The experiment was optimized for 4 Hz rather than 10 Hz based on the difficulty we noted in observing $^3J_{NH}$ couplings from peri-protons to the N9 amide nitrogen in the *Strychnos* alkaloids.[3] The data were acquired as 4096 × (96 × 2) hypercomplex files with a total of 448 transients accumulated/t_1 increment. Pulse widths were 9.8 μs for ^1H and 30.0 μs for ^{15}N at powers of 56 and 63 dB (63 dB maximum). The F_1 spectral window employed was set from 100−300 ppm to accommodate a plausible range for the N10 indole ^{15}N chemical shift. Gradient pairs were optimized as 5:5:±1 (10 000:10 000:±2010) for ^{15}N.[4] Long-range correlations observed from the aromatic resonances to the two nitrogens in the molecule after a 42 h data accumulation are shown in Figure 2. Correlations were observed from H4, the *N*-methyl singlet, and the H11 singlet to the dihydroquinoline N5 resonance at 154.7 ppm within 6 h of initiating data accumulation. In contrast, the couplings from the H9 and H11 resonances to the indole N9 resonating at 207.8 ppm were much weaker and were still only barely visible in the spectrum after 42 h. Chemical shifts for the two ^{15}N resonances of **1** are quite reasonable based on the reported chemical shifts of the ^{15}N resonances of **2**.[5] In a series of analogs related to **2**, pyrrole ^{15}N shifts in which the double bond was exo to the pyridine ring ranged from 199.6 to 206 ppm; dihydropyridine *N*-methyl ^{15}N shifts were more variable and ranged from 147.3 to 170.4 ppm.



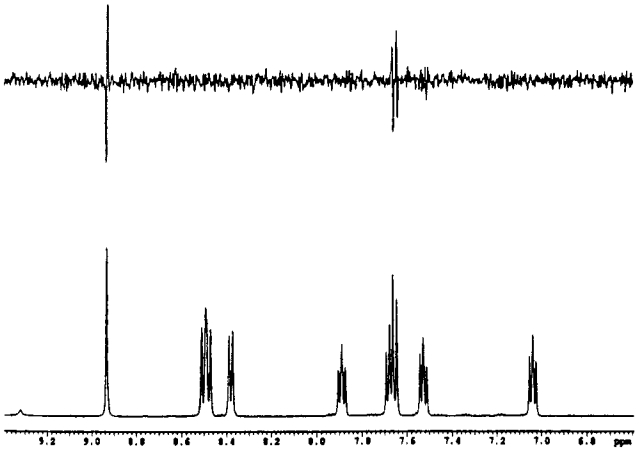


Figure 3. ^1H reference spectrum (bottom) and slice through F_2 at the chemical shift of N10 (208.7 ppm) showing the antiphase nature of long-range $^1H-^{15}N$ correlations from H9 and H11.

Long-range $^1H-^{15}N$ couplings were measured by zero-filling the data to 8K points in F_2 and phasing individual ^{15}N traces in F_1. Slices through F_2 were then phased to afford antiphase doublets for the resonances which would normally appear as singlets in the proton reference spectrum as shown in Figure 3. Since $^1H-^{15}N$ couplings are not refocused in the experiment following reconversion to observable ^1H magnetization,[1] the $^1H-^{15}N$ coupling is measured as the separation between the antiphase limbs of the multiplet. As will be noted from **1**, all of the long-range couplings to both N5 and N10 in the spectrum of cryptolepine (**1**) are less than 4 Hz. Thus, it is not particularly surprising that couplings to N10 were not observed in our earlier nongradient experiments since those spectra were optimized for 10 Hz.

In conclusion, the acquisition of long-range $^1H-^{15}N$ GHMQC spectra provides a viable means of beginning to utilize ^{15}N in strategies to elucidate the structures of potentially novel alkaloids when reasonable samples of material are available. By combining the experiment we have described with gradient micro inverse-detection probes, which are now available (gradient micro inverse-detection probes (IDG-500-3 and IDTG-500-3) are currently available from Nalorac Crogenics Corp., Martinez, CA), it should be possible to push sample requirements substantially below those utilized in the present work making the utilization of ^{15}N as a structural probe even more viable. We have already demonstrated that it is feasible to acquire $^1H-^{15}N$ direct correlation spectra with an 800 μg sample (~3.7 μmol) of the alkaloid quindolinone overnight using a nongradient experiment (*6*). By employing long-range $^1H-^{15}N$ GHMQC in conjunction with a gradient micro inverse probe, we anticipate that it should be possible to reduce sample requirements by a factor of as much as 4 while still maintaining reasonable acquisition times (over a weekend or shorter).

References and Notes

(1) Martin, G. E.; Crouch, R. C.; Sharaf, M. H. M.; Schiff, P. L. Jr. *$^1H-^{15}N$ Direct and Long-Range Heteronuclear Shift Correlation Techniques—Potential Applications*, 34th Annual Meeting of the American Society of Pharmacognosy, San Diego, CA, July 1993; poster 101.
(2) Martin, G. E.; Crouch, R. C. *J. Heterocycl. Chem.* **1995**, *32*, 1665−1669.

(3) Martin, G. E.; Crouch, R. C.; Andrews, C. W. *J. Heterocycl. Chem.* **1995**, *32*, 1759−1766.
(4) Ruiz-Cabello, J.; Vuister, G. W.; Moonen, C. T. W.; van Geldern, P.; Cohen, J. S.; van Zilj, P. C. M. *J. Magn. Reson.* **1992**, *100*, 282−302.
(5) Kaczmarek, L.; Nantka-Namirski, P.; Stefaniak, L.; Webb, G. A.; Davoust, D.; Basselier, J. J. *Magn. Reson. Chem.* **1985**, *23*, 853−855.
(6) Crouch, R. C.; Davis, A. O.; Spitzer, T. D.; Martin, G. E.; Sharaf, M. H. M.; Schiff, P. L., Jr.; Phoebe, C. W., Jr.; Tackie, A. N. *J. Heterocycl. Chem.* **1995**, *32*, 1077−1080.

NP9600412