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Low-Level Long-Range $^{1}H-^{15}N$ Heteronuclear Shift Correlation at Natural Abundance Using Submicro NMR Techniques

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Abstract: Gradient inverse-detected NMR methods have provided experimental access to long-range $^{1}H-^{15}N$ heteronuclear shift correlation data at natural abundance. When used in conjunction with newly developed submicro-inverse-detection gradient (SMIDG) NMR probes, sample size requirements are dramatically reduced. Long-range $^{1}H-^{15}N$ GHMBC data are shown for a 1 mg sample (~3 μmol, 0.11 M) of the alkaloid strychnine (**1**) dissolved in 30 μL of $CDCl_3$. All of the correlations observed in previous studies using samples in the range of 20−40 mg in 5 mm NMR probes were observed in data acquired overnight on a 1 mg sample in a 1.7 mm SMIDG NMR probe. A previously unreported long-range correlation, $^{3}J_{H-15a-N(19)}$, was observed in data recorded over a weekend for the 1 mg sample. Data recorded over a weekend using a 1 μmol (334 μg) sample of strychnine (**1**) in 30 μL of $CDCl_3$ contained the majority of the responses from the 1 mg sample. The practical limit of detection for long-range $^{1}H-^{15}N$ heteronuclear shift correlation data at natural abundance would appear to be ~1 μmol with data acquisition over a weekend or longer using present submicro-NMR probe technology.

Long-range $^{1}H-^{15}N$ heteronuclear shift correlation studies at natural abundance represent an area of active research interest, with a growing number of applications being reported. There have been numerous applications reported for alkaloids,[1−16] an area of focus where the application of long-range $^{1}H-^{15}N$ heteronuclear shift correlation is obvious. Widespread availability of instruments with pulsed field gradient (PFG) capabilities has facilitated these studies largely through the suppression of t_1 noise in gradient, heteronuclear NMR experiments. Unfortunately, the majority of data now being reported have depended upon relatively large samples and 5 mm probe technology, with sample volumes ranging from 300 to 600 μL. Working concentrations can be maintained with a concurrent reduction in sample size, however, by resorting to 3 mm micro-NMR probes. Typical working sample volumes range from 120 to 150 μL for a standard 3 mm NMR tube down to as little as 70−80 μL if an investigator is willing to use a specialized Shigemi NMR microtube, with the commensurate increase in shimming overhead.[17,18] A still further significant reduction in sample volume can be realized by resorting to the newly reported SMIDG (submicro-inverse-detection gradient) 1.7 mm NMR probes, the tubes for which use a sample volume in the range of 25−30 μL.[19−21] We report here the first results obtained using this probe technology for $^{1}H-^{15}N$ heteronuclear shift correlation.[22]

To evaluate the capability of SMIDG NMR probe technology for the acquisition of $^{1}H-^{15}N$ heteronuclear shift correlation data at natural abundance, we elected to reexamine strychnine (**1**), which has been the subject of two previous reports.[1,3] The earlier of the previous studies, that of Koshino and Uzawa,[1] employed a sample of 40 mg of **1** dissolved in 300 μL $CDCl_3$ (0.42 M). The latter study by one of the present authors[3] used a more modest 20 mg of **1** dissolved in 500 μL of $CDCl_3$ (0.13 M). For the present study, we elected to begin by dissolving 1 mg of **1** (~3 μmol) in 30 μL of $CDCl_3$, affording a 0.11 M solution comparable to that used in our previous study, albeit with substantially reduced sample volume and a commensurate reduction in the quantity of alkaloid. The sample was transferred to a

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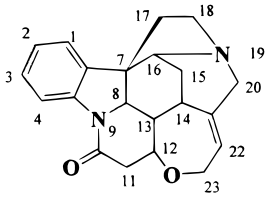
1.7 mm SMIDG NMR tube and sealed. Data were acquired using a Varian INOVA 600 NMR spectrometer operating at 599.75 MHz for ^{1}H observation and equipped with a Nalorac SMIDG-600 1.7 mm NMR probe. The experiment was performed using a standard GHMBC pulse sequence with gradient ratios optimized for ^{15}N (5:1 with actual gradient strengths of 10.00 and 2.02 G cm^{-1}).[23] The long-range delay in the experiment was optimized for an assumed 10 Hz coupling (50 ms). The data were acquired as 4096 × 40 States-TPPI files and were linear predicted to 120 files in t_1, followed by zero-filling to afford the final 4096 × 512 point spectrum shown in Figure 1. The data were processed using sine-bell multiplication prior to the first Fourier transform and cosine multiplication prior to the second, the time constants optimized to the acquisition time in both frequency domains. Spectral widths were 5192 and 8509 Hz in F_2 and F_1, respectively. Pulse widths for ^{1}H and ^{15}N were 6.65 and 21.1 μs, respectively, at power levels of 48 and 59 dB, respectively (maximum 63 dB). Data were acquired in 18 h with 512 transients accumulated/t_1 increment. The time/transient was 1.394 s (1.0 s interpulse + acquisition time). Individual slices at the ^{15}N chemical shift of the two nitrogens are plotted above the proton reference spectrum in Figure 2. The measured signal-to-noise ratio (S/N) for the spectrum was 32:1; the noise region was arbitrarily defined as the 600 Hz segment of the spectrum from 4.8 to 5.8 ppm in the trace plotted for N(19).



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Correlations observed in the spectrum shown in Figure 1 are comparable to those reported in the two previous studies.[1,3] In part, the performance can be attributed to the concentration, which was comparable to our earlier 500 MHz work using a 5 mm inverse detection probe. More pragmatically, 1.7 mm SMIDG NMR probe technology brings the acquisition of ^{1}H−^{15}N long-range heteronuclear shift correlation data at natural abundance down to a reasonable sample size of several micromoles. Such sample quantities are much more readily available than the tens of micromoles required when these experiments are to be performed using conventional 5 mm probe technology.

The nitrogen resonances in the present study were observed at 35.1 and 149.1 ppm. Both nitrogens are shifted downfield only slightly relative to our previous study,[3] in which they were observed at 35.0 and 148.0 ppm, respectively. As the concentration was essentially the same in both studies and the solvent identical, the differences can most probably be attributed to the difference in temperature; our earlier work was done at 25 °C while the present study was performed at 35 °C. Data reported by Koshino and Uzawa[1] place the nitrogen chemical shifts somewhat upfield of this and our previous study by approximately 10 ppm, although exact shifts were not reported. It should be noted,

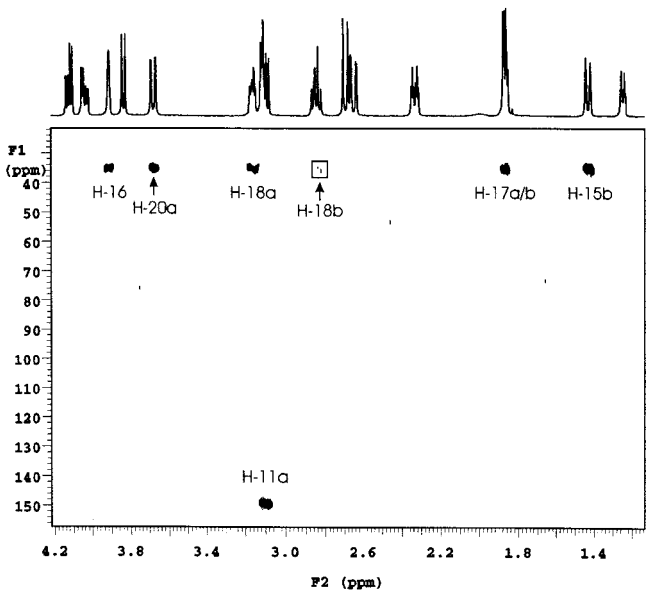

Figure 1. ^{1}H−^{15}N GHMBC spectrum of 1 mg (~3 μmol, 0.11 M) of strychnine (**1**) at natural abundance in 30 μL of $CDCl_3$. The experiment was optimized for an assumed 10 Hz (50 ms) long-range coupling. The high-resolution proton spectrum is plotted atop the contour plot. Data acquisition parameters are specified in the text. Total acquisition time was 18 h.

however, that their study referenced to the ^{15}N shift of $^{15}NH_4NO_3$, while this and our previous study[3] were referenced to the calculated shift of ammonia relative to nitromethane. The difference in referencing schemes and changes in temperature could account for the difference in observed chemical shifts.

Individual long-range responses to N(9) and N(19) and their intensities are shown in the stacked slice summary presented as Figure 2. Correlations to N(9) included responses from H-8 and H-13 (3.84 and 1.24 ppm, respectively), both of which were weak. The response correlating H-11a to N(9) at 3.12 ppm was quite intense and readily visible in the contour plot presented in Figure 1. If these were data for an unknown, care would necessarily have to be exercised in the utilization of the responses correlating both H-8 and H-13 to N(9). These responses are sufficiently weak that only the breadth of the response when the data are viewed as the slice allows them to be considered as viable correlations to N(9). This is particularly true of the H-13-N(9) correlation response, which is considerably weaker than the response from H-8.

The long-range couplings to N(19) are more numerous and included most of the protons within two or three bonds of this nitrogen. The weakest two correlations to N(19) are those from H-20b (2.69 ppm) and H-18b (2.84 ppm). The former is not visible in the contour plot at all, the latter is only weakly visible. Other more intense correlations to N(19) observed in the contour plot presented in Figure 1 include the following: H-16 (3.91 ppm), H-20a (3.68 ppm), H-18a (3.16 ppm), H-17a/b (1.86 ppm), and H-15b (1.42 ppm). All of the observed correlations to N(19) in the GHMBC spectrum acquired overnight would be considered reliable and could be employed with confidence in the elucidation of an unknown structure.

The acquisition of a ^{1}H−^{15}N GHMBC spectrum of strychnine over a weekend (64 h, see Figure 3) using

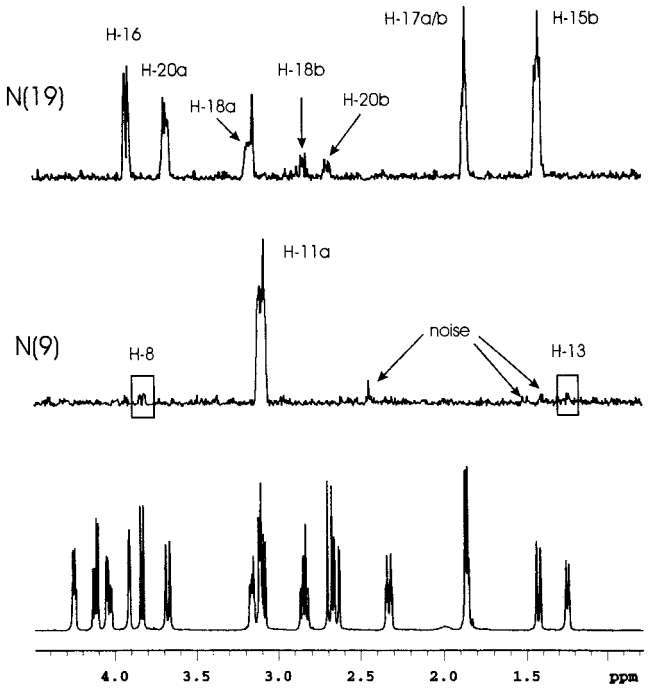

Figure 2. 600 MHz ^{1}H NMR reference spectrum of 1 mg (~3 μmol, 0.11 M) of strychnine (**1**) in 30 μL of $CDCl_3$ (bottom trace); slice taken at 149.1 ppm corresponding to the ^{15}N shift of N(9) from the 10 Hz optimized ^{1}H−^{15}N GHMBC spectrum shown in Figure 1 (middle trace); slice taken at 35.1 ppm corresponding to the ^{15}N shift of N(19) from the 10 Hz optimized ^{1}H−^{15}N GHMBC spectrum shown in Figure 1 (top trace). Data were acquired overnight (18 h). The low intensity of the H-8 and H-13 (boxed) correlations to N(9) would call question to their authenticity, particularly for H-13. In such an instance, the acquisition of data over a longer period of time (compare the intensity of the H-8 and H-13 responses in a weekend acquisition (64 h) shown in Figure 3) or a selective 1D ^{1}H−^{15}N spectrum would be required to confirm these responses. It should also be noted, in the case of the H-8 response, that the width of the limbs of the multiplet in the trace is consistent with a legitimate correlation rather than noise, the latter generally appearing as an intense spike with no "width" associated with it.

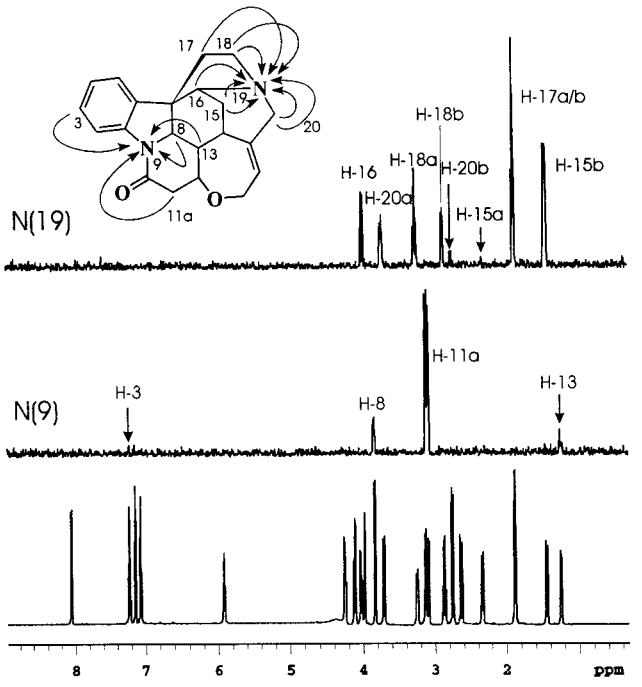

Figure 3. 600 MHz ^{1}H NMR reference spectrum of 1 mg (~3 μmol, 0.11 M) of strychnine (**1**) in 30 μL of $CDCl_3$ (bottom trace); slice taken at 149.1 ppm corresponding to the ^{15}N shift of N(9) from the 10 Hz optimized ^{1}H−^{15}N GHMBC spectrum shown in Figure 1 (middle trace); weak responses denoted by arrows were either weakly or not visible in the contour of these data; slice taken at 35.1 ppm corresponding to the ^{15}N shift of N(19) from the 10 Hz optimized ^{1}H−^{15}N GHMBC spectrum shown in Figure 1 (top trace). The data from the two-dimensional NMR experiment were from a weekend acquisition (64 h). The very weak correlation in the middle trace from H-3 to N(9) was confirmed in the data published by Koshino and Uzawa[1] that were acquired on a 40 mg smaple.

the 1 mg/30 μL sample gave, as expected, roughly double the s/n ratio of the data shown in Figures 1 and 2. The measured S/N ratio was 62:1, which is reasonable since the acquisition was roughly four times the duration of the overnight data shown in Figures 1 and 2. We show these data for two primary reasons. First, the improved response intensity, particularly for the H-8 and H-13 responses, confirms the reliability of these correlations, and a weekend acquisition is certainly not unreasonable in duration. Second, a three-bond long-range correlation from H-15a to N(19) is observed in the weekend data for the first time.

Finally, in an effort to push the limits of detection, a GHMBC spectrum was acquired on a sample containing 1 μmol (330 μg) of strychnine dissolved in 30 μL of $CDCl_3$ (0.037 M). The spectrum was acquired over a weekend (65 h), affording the data shown in Figure 4 as slices plotted above a high-resolution reference spectrum. The three most intense resonances in the spectrum, the H-17a/b and H-15b correlations to N(19) and the H-11b correlation to N(19), were visible in the contour plot. When the slice taken at the chemical shift of N(19) was examined, responses below the threshold used to prepare the contour plot were observed from H-16, H-20a, H-18a, and H-18b. For N(9), a weak response was observed in the slice from H-8, but not from H-13.

In conclusion, the availability of 1.7 mm SMIDG NMR probe technology makes it quite reasonable and practical to consider employing long-range ^{1}H−^{15}N heteronuclear shift correlation experiments in the assembly of novel alkaloid and chemical structures. Usable data can be acquired overnight on ~3 μmol of modest molecular weight compounds, provided that the investigator is willing to utilize responses from slices plotted at the chemical shifts of the individual nitrogens. Acquiring the data over a weekend (64 h) yielded high-quality data, as shown by Figure 3. Finally, we have also demonstrated that it is possible to work at the 1 μmol level provided that a minimum of a weekend is available. At this level, which we presently feel represents the reasonable limit of detection for ^{1}H−^{15}N long-range heteronuclear shift correlation experiments at natural abundance, strong reliance must be placed on the information contained in the individual slices taken at the nitrogen chemical shifts. The intensity of the responses being utilized from the data generated at this level will obviously determine or limit the confidence that one has in the interpretation and the structures that result from the utilization of the data. Individuals required to work at these levels as a result of severely restricted samples would probably do well to consider the acquisition of selective 1D ^{1}H−^{15}N heteronuclear shift correlation spectra once the chemical shifts of the

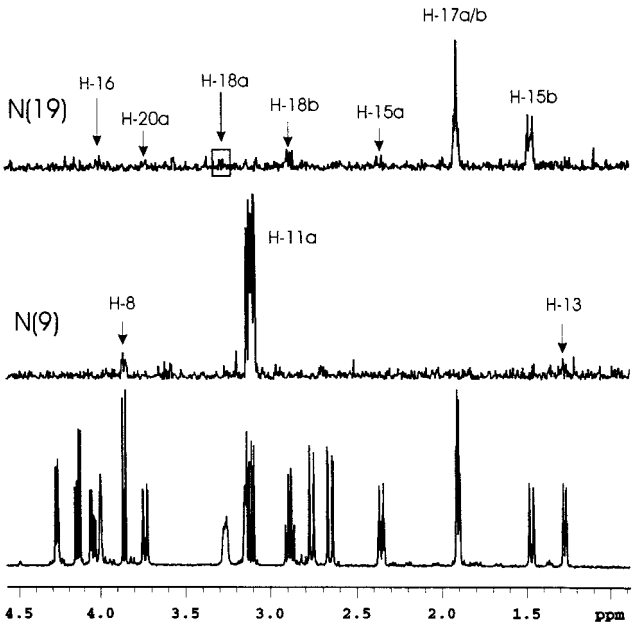

Figure 4. 600 MHz ^{1}H NMR reference spectrum of 334 μg (1 μmol, 0.037 M) of strychnine (**1**) in 30 μL of $CDCl_3$ (bottom trace); slice taken at 149.1 ppm corresponding to the ^{15}N shift of N(9) from the 10 Hz optimized ^{1}H−^{15}N GHMBC spectrum shown in Figure 3 (middle trace); slice taken at 35.1 ppm corresponding to the ^{15}N shift of N(19) from the 10 Hz optimized ^{1}H−^{15}N GHMBC spectrum shown in Figure 3 (top trace). The H-16 and H-20a responses are quite weak but were readily discernible on the basis of the width of the response. The H-18a response is also weak and is enclosed in the boxed region of the top trace. In dealing with the structure of an unknown, the responses for H-16 and H-20a would perhaps be usable with reservation; we would be inclined not to employ or consider the response from H-18a in determining the structure of an unknown. The H-18a response is shown here simply because we were certain of its presence in the spectrum on the basis of the previous work[1,3] and the higher concentration data acquired in the present study. It might be possible, if this were an unknown, to utilize the H-18a response by watching the behavior of the line width as a function of successively reprocessing the data with varied weighting parameters. Pragmatically, it would be obvious from an ^{1}H−^{13}C GHSQC spectrum that H-18a/b were anisochronous methylene protons. This knowledge obviates the need to use the information provided by the H-18a−N(19) correlation. The slice data were acquired over a weekend (65 h).

nitrogens have been reliably established from 2D NMR experiments. Using this approach, one or as many one-dimensional experiments as necessary can be performed with proportionately higher signal-to-noise ratios than would be attainable in the 2D experiment. This approach, using an experiment given the acronym SIMBA by one of the authors,[24] has been demonstrated on the alkaloid cryptospirolepine for a weak ^{1}H−^{13}C correlation.[25] Extending this experiment to ^{1}H−^{15}N is a straightforward undertaking and is not terribly different from the proton-selected 2D ^{1}H−^{15}N GHNMBC experiment that has been successfully applied in a recently reported study of the drug delavirdine.[26]

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