Optimization of Long-Range ^{1}H-^{15}N 2D NMR Experiments at Natural Abundance -- Assignment of the ^{15}N Resonances of Cryptospirolepine at the Submilligram Level

Chad E. Hadden and Gary E. Martin*

Rapid Structure Characterization Group, Pharmaceutical Development, Pharmacia & Upjohn, Kalamazoo, MI 49001-0199

Albert N. Tackie

Center for Scientific Research into Plant Medicine, Mampong-Akwapim, Ghana

Paul L. Schiff, Jr.

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

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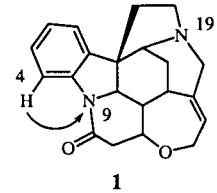
Two-dimensional nmr methods have had an undeniable influence on our ability to elucidate complex chemical structures, as attested by the number of published papers and monographs on the subject now extant in the literature [1]. Heteronuclear shift correlation methods, in general, have perhaps had the greatest impact. Long-range chemical shift correlation experiments have had a particularly profound influencce on the elucidation of chemical structures beginning from the first, unrealized suggestion for this category of experiment in 1980 [2], followed by the experimental demonstration of this powerful technique in the work of Reynolds in 1983 [3]. Inverse-detected long-range ^{1}H-^{13}C heteronuclear shift correlation, in the form of the HMBC experiment first described in 1986 [4], increased the breadth of application of these experiments by virtue of the substantial reduction in sample size afforded by utilizing proton rather than heteronucleus detection. Further gains were afforded by the work of Hurd and co-workers who reported the development of gradient-enhanced versions of the direct, long-range, and relayed coherence transfer heteronuclear shift correlation experiments in 1991 [5-7]. It was not until 1993 that the first posters were presented reporting the extension of HMBC experiments to include ^{1}H-^{15}N correlation at natural abundance [8,9], the first published reports appearing in 1995 [10-12]. In the intervening scant three years, in excess of 50 papers reporting the utilization of long-range ^{1}H-^{15}N experiments at natural abundance have now appeared and form the topic of a recent review [13].

With any long-range heteronuclear shift correlation experiment, one of the challenges to the spectroscopist or chemist performing the experiment is the optimization of the delays used for the long-range transfer of magnetization from proton to the heteronuclide for chemical shift labeling during evolution followed by transfer back to the proton(s) for detection. For ^{1}H-^{13}C long-range heteronuclear correlation experiments, this is a relatively simple task; optimization for long-range couplings from 6 to 10 Hz generally provides acceptable results. In the case of ^{1}H-^{15}N correlation experiments, the optimization of the experiment is somewhat less clear cut. In our experience, long-range ^{1}H-^{15}N couplings range from ~2 Hz or less to 16 Hz or more [11]. A particularly difficult scenario arises when dealing with "*ortho*" couplings from an aromatic proton to a nitrogen not contained in the same ring, as in the case of the correlation from H4 to N9 of strychnine (**1**) [11,12] or the coupling of H9 or H11 to N10 of cryptolepine (**2**) [14]. Generally, these couplings are difficult to observe, if they are observed at all.



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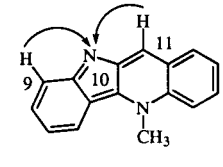

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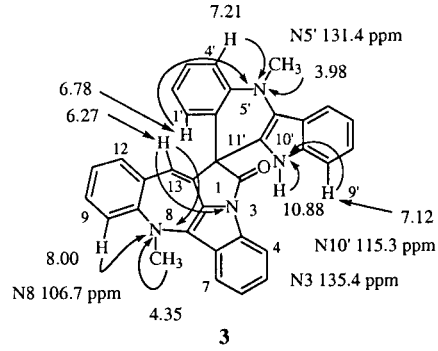
In the case of the complex spiro nonacyclic alkaloid cryptospirolepine (**3**), whose structure was first elucidated in 1993 [15], three of the four nitrogens have correlations to them which render them readily observed [16]. In the initial elucidation of the structure, Martin and co-workers [15] reported data for only the protonated indole nitrogen, N10'. The observation of direct ^{1}H-^{15}N correlation responses is a facile undertaking, providing that the proton on the nitrogen is not in exchange or involved in autoprotonation elsewhere in the molecular structure [17]. A ^{1}H-^{15}N HMQC spectrum was acquired overnight, locating this nitrogen at 115.3 ppm using a 2.5 mg sample of **3** dissolved in 600 µl of dimethyl-d_6 sulfoxide. The data were acquired using a 500 MHz instrument equipped with a standard 5 mm inverse-detection probe. More recently, cryptospirolepine (**3**) has

been employed as a model compound [16] to demonstrate the rapid acquisition of heteronuclear shift correlation data using submicro (SMIDG) nmr probe technology [18-20]. As a part of this study, the acquisition of direct and long-range ^1H-^{15}N spectra were reported at 600 MHz using a 1.5 μmole (~750 μg) sample of the alkaloid dissolved in 30 μl of dimethyl-d_6 sulfoxide. The indole N10'-H direct correlation was observable on this sample using this probe hardware in <50 min with > 15:1 signal-to-noise in a GHSQC spectrum. An overnight (18 hours) GHMBC experiment optimized for 3 Hz afforded correlations to the two *N*-methyl nitrogen resonances contained in the molecule, N8 and N5'. The optimization was selected based on the measured 3.6 Hz coupling of H9 to N10 in cryptolepine (**2**). As expected, correlations were observed from the methyl groups to their respective nitrogens. In addition, the ortho correlations from H9 to N8 and from H4' to N5' were also obseved in this spectrum. The correlation from H4 to the amide N3 resonance was not observed, nor was a correlation from H9' to N10'. These correlations were also not observed in 6 Hz optimized spectrum acquired over a weekend.

Early work of Koshino and co-workers [12] has, however, demonstrated the feasability of observing four-bond ($^4J_{NH}$) long-range correlations to nitrogen in heteroaromatic systems by resorting to optimization of the long-range delays in the ^1H-^{15}N GHMBC experiment to <2 Hz. Using a 65 mg sample of 1,2,4-triazolo[1,5-*a*]pyrimidine, a long-range experiment was performed in which the long-range delays were optimized for ~1.7 Hz (300 ms). All possible long-range correlations to nitrogen, including several four-bond correlations, were observed in these data. It was on the basis of Koshino's work [12] that we resorted to a 2.5 Hz (200 msec) optimization, recording the spectrum shown in Figure 1. Because of the enormous difference in the size of the sample in our present work and that previously used by Koshino (750 μg *vs.* 65 mg), our acquisition time was substantially longer than that which he reported, despite the sensitivity advantages conferred by using the submicro nmr probe at 600 MHz.

New correlations were observed in the 200 ms optimized experiment for four-bond correlations from the H13 vinyl proton resonating at 6.62 ppm to both the amide nitrogen, N3, which resonates at 135.4 ppm and to the N8 azepine nitrogen resonating at 106.7 ppm. The former establishes the chemical shift of the N3 resonance for the first time. A four-bond correlation was also observed from H1' resonating at 6.78 ppm to N5', which resonates at 131.4 ppm. No trace of a correlation from H4 to the N3 amide nitrogen was observed in this experiment despite the 2.5 Hz optimization. A new ortho correlation was, however, observed from the H9' proton resonating at 7.12 ppm to the protonated indole N10' nitrogen resonance at 115.3 ppm.



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Obviously, it would be desirable to eliminate the ambiguities of optimizing the long-range delays when performing ^1H-^{15}N GHMBC experiments at natural abundance since these experiments can be relatively time-consuming. An experiment recently described by Berger and co-workers, ACCORD-HMBC [21], offers the potential to solve this type of problem. In the ACCORD-HMBC experiment, the long-range delay is successively decremented from one increment of the evolution period to the next, providing uniform or "accordion" excitation [22,23].

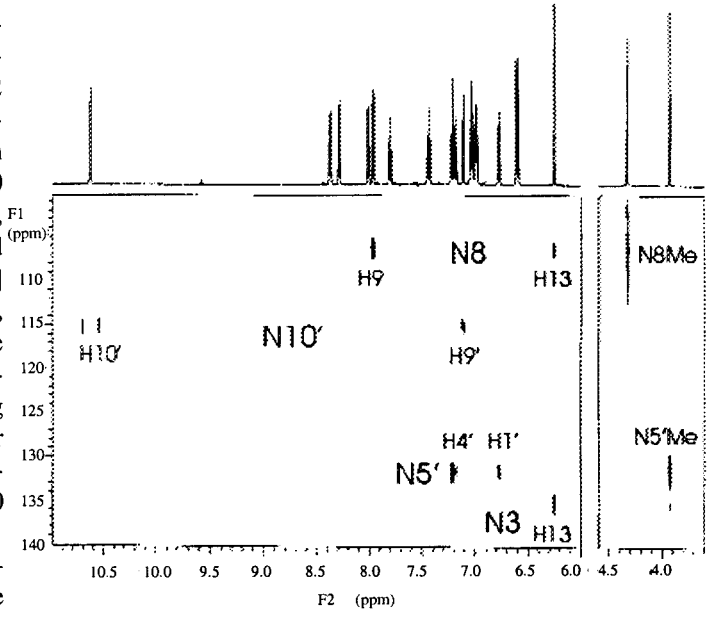

Figure 1. Long-range ^1H-^{15}N GHMBC spectrum of cryptospirolepine (**3**) recorded at 600 MHz. The exeperiment was performed using a Varian *INOVA* 600 equipped with a Nalorac SMIDG-600-1.7 submicro nmr probe. The sample used was prepared by dissolving 750 μg (~1.5 mmole) of **3** in 30 μl of dimethyl-d_6 sulfoxide. The long-range delay was optimized for 2.5 Hz (200 ms). The data were acquired as 4096 x 32 States-TPPI files with 8446 transients accumulated/t_1 increment. The F_1 spectral window was 100-155 ppm. The data were linear predicted to 96 files in t_1 and zero-filled to 128 points during processing. Data were processed using shifted sinebell and cosine multiplication prior to the first and second Fourier transformations. The vertical scale for the methyl region of the spectrum, shown in the right panel, was set to 25% of the vertical scale used for the aromatic region because of the intensity of the *N*-methyl correlations.

We are currently investigating the application of this experiment to long-range ^{1}H-^{15}N data acquisition which will form the subject of a forthcoming report.

REFERENCES AND NOTES

[1] Any survey of published mansucripts is now virtually impossible, the number of references to papers involving 2D nmr is now well in the 1000's. Selected examples of the now numerous monographs that have been published include the following as representative examples: A. Bax. Two-Dimensional NMR in Liquids, D. Reidel Publishing Co., Boston, 1982; R. R. Ernst, G. Bodenhausen, and A. Wokaun, Principles of Nuclear Magnetic Resonance in One and Two Dimensions, Clarendon, Press, Oxford, 1987; W. S. Brey, Pulse Methods in 1D and 2D Liquid-Phase NMR, Academic Press, San Diego, 1988; G. E. Martin and A. S. Zektzer, Two-Dimensional NMR Methods for Establishing Molecular Connectivity -- A Chemist's Guide to Experiment Selection, Performance and Interpretation, VCH, New York, 1989; K. Nakanishi, One-Dimensional and Two-Dimensinal NMR Spectra by Modern Pulse Techniques, University Science Books, Mill Valley, CA, 1990; W. R. Croasmun and R. M. K. Carlson, Two-Dimensional NMR Spectroscopy -- Applications for Chemists and Biochemists, 2nd ed., VCH, New York, 1994.

[2] K. Hallenga and G. van Binst, *Bull. Magn. Reson.*, **2**, 343 (1980).

[3] W. F. Reynolds, R. G. Enriquez, L. I. Escobar, and X. Lozoya, *Can. J. Chem.*, **62**, 2421 (1984).

[4] A. Bax and M. F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).

[5] R. E. Hurd and B. K. John, *J. Magn. Reson.*, **91**, 648 (1991).

[6] R. E. Hurd and B. K. John, *J. Magn. Reson.*, **92**, 658 (1991).

[7] B. K. John, D. Plant, S. L. Heald, and R. E. Hurd, *J. Magn. Reson.*, **94**, 664 (1991).

[8] G. E. Martin, R. C. Crouch, M. H. M. Sharaf, and P. L. Schiff, Jr., 34th Ann. Mtg. Am. Soc. Pharmacog., San Diego, CA, 1993, poster #101.

[9] J. Uzawa, H. Utsumi, H. Koshino, T. Hinomoto, and K. Anzai, Abstr. 32nd NMR Conference, Tokyo, 1993, pp. 147-150.

[10] R. C. Crouch and G. E. Martin, *J. Heterocyclic Chem.*, **32**, 1665 (1995).

[11] G. E. Martin, R. C. Crouch, and C. W. Andrews, *J. Heterocyclic Chem.*, **32**, 1759 (1995).

[12] H. Koshino and J. Uzawa, *Kagaku to Seibutsu*, **33**, 252 (1995).

[13] G. E. Martin and C. E. Hadden, *J. Nat. Prod.*, submitted (1999).

[14] G. E. Martin, R. C. Crouch, M. H. M. Sharaf, and P. L. Schiff, Jr., *J. Nat. Prod*, **59**, 2 (1996).

[15] A. N. Tackie, G. L. Boye, M. H. M. Sharaf, P. L. Schiff, Jr., R. C. Crouch, T. D. Spitzer, R. L. Johnson, J. Dunn, D. Minick, and G. E. Martin, *J. Nat. Prod*, **56**, 653 (1993).

[16] G. E. Martin, C. E. Hadden, A. N. Tackie, M. H. M. Sharaf, and P. L. Schiff, Jr., *Magn. Reson. Chem.*, **37**, 527 (1999).

[17] K. A. Farley, G. S. Walker, and G. E. Martin, *Magn. Reson. Chem.*, **35**, 671 (1997).

[18] G. E. Martin, R. C. Crouch, and A. P. Zens, *Magn. Reson. Chem.*, **36**, 551 (1998).

[19] G. E. Martin, J. E. Guido, R. H. Robins, M. H. M. Sharaf, P. L. Schiff, Jr., and A. N. Tackie, *J. Nat. Prod*, **61**, 555 (1998).

[20] C. E. Hadden and G. E. Martin, *J. Nat. Prod*, **61**, 969 (1998).

[21] R. Wagner and S. Berger, *Magn. Reson. Chem.*, **36**, S44 (1998).

[22] G. Bodenhausen and R. R. Ernst, *J. Am. Chem. Soc.*, 104, 1304 (1982).

[23] G. Kontaxis and J. Keeler, *J. Magn. Reson.*, **A115**, 35 (1995).