Long-Range ^{1}H-^{15}N Two-Dimensional Heteronuclear Shift Correlation of the Alkaloid Vincamine at Natural Abundance

Gary E. Martin

Pharmacia & Upjohn, Pharmaceutical Development, Kalamazoo, MI 49001-0199

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Long-range ^{1}H-^{15}N heteronuclear correlation pathways in the alkaloid vincamine, a structural constituent or biogenetic congener of numerous *Vinca* alkaloids, are reported. Correlations were observed through the use of the GHNMQC (Gradient-enhanced Hydrogen-Nitrogen Multiple Quantum Coherence) 2D nmr experiment at natural abundance. An unanticipated four-bond coupling between the H15a resonance and N4 was observed in contrast to the typical two- and three-bond coupling pathways. Nitrogen-15 chemical shift comparisons are drawn between vincamine and structurally related alkaloids including ajmaline and the velbanamine portion of the semi-synthetic alkaloid vinorelbine (Navelbine™).

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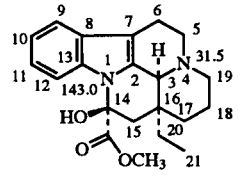
Sir:

Nitrogen-15 has been largely ignored by natural products chemists as a nuclide to be used in structure characterization studies because of its inherent insensitivity, a function of a low gyromagnetic ratio coupled with low relative abundance. Despite these limitations, we have been able to demonstrate [1-5] that ^{1}H-^{15}N heteronuclear correlations can be established at natural abundance on samples consisting of a few millimoles using conventional 5 mm gradient inverse or triple resonance probes through the use of an experiment we have recently given the acronym GHNMQC (Gradient Hydrogen-Nitrogen Multiple Quantum Coherence) [5]. The acronym was selected since the experiment is derived from components of the HMQC [6] and its experimental predecessors [7,8], the HMBC [9] experiment, and their gradient enhanced counterparts [10,11].

The ^{1}H-^{15}N long-range connectivities of the alkaloid vincamine (**1**) are now reported. Vincamine was selected as a desireable candidate for study to extend the range of our previous studies since it, or closely related biogenetic congeners, are a structural constituent of numerous other more complex alkaloids, for example the bis-indole alkaloids of the *Vinca* family, which include well known medicinally useful alkaloids such as vincristine and vinblastine as well as numerous others.

Examining the structure, based on our previous work, we anticipated that only protons within two- or three-bonds of each of the nitrogens would be long-range coupled to their respective nitrogen. The minimized structure of the molecule is shown by **2**; the structure is oriented to be viewed from the top face of the molecule to afford a presentation analogous to the conventional chemical structure shown by **1**.

Before we can embark on an examination and assignment of the long-range ^{1}H-^{15}N coupling pathways of vincamine, we must first have in hand a set of reliable resonance assignments for the molecule. Proton resonance



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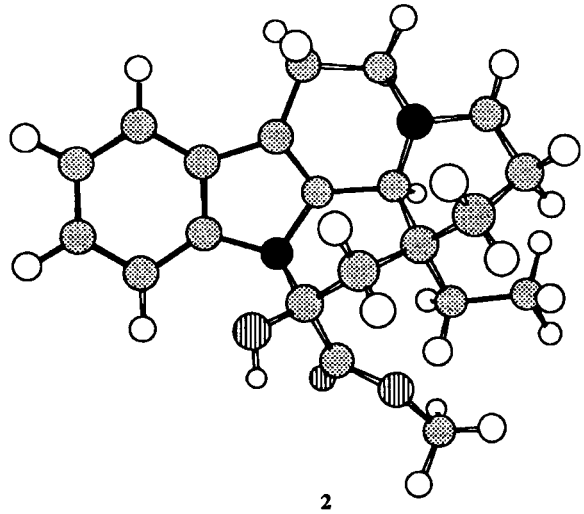

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assignments for vincamine (**1**) in deuteriochloroform have been reported by several research groups [12-15]. Since deuteriodimethyl sulfoxide was used as a solvent to dissolve the 15 mg sample used for the long-range proton-nitrogen nmr experiment coupled with a desire to be able to observe the hydroxyl proton, the proton resonance assignments for the molecule were established at 500 MHz using IDR-GHMQC-TOCSY [16,17] data acquired with a mixing time of 18 msec. Confirmatory COSY and GHMQCPS spectra were recorded, but were unnecessary for the assignment.

The IDR-GHMQC-TOCSY spectrum of the aliphatic region of **1** is shown in Figure 1. Inverted direct

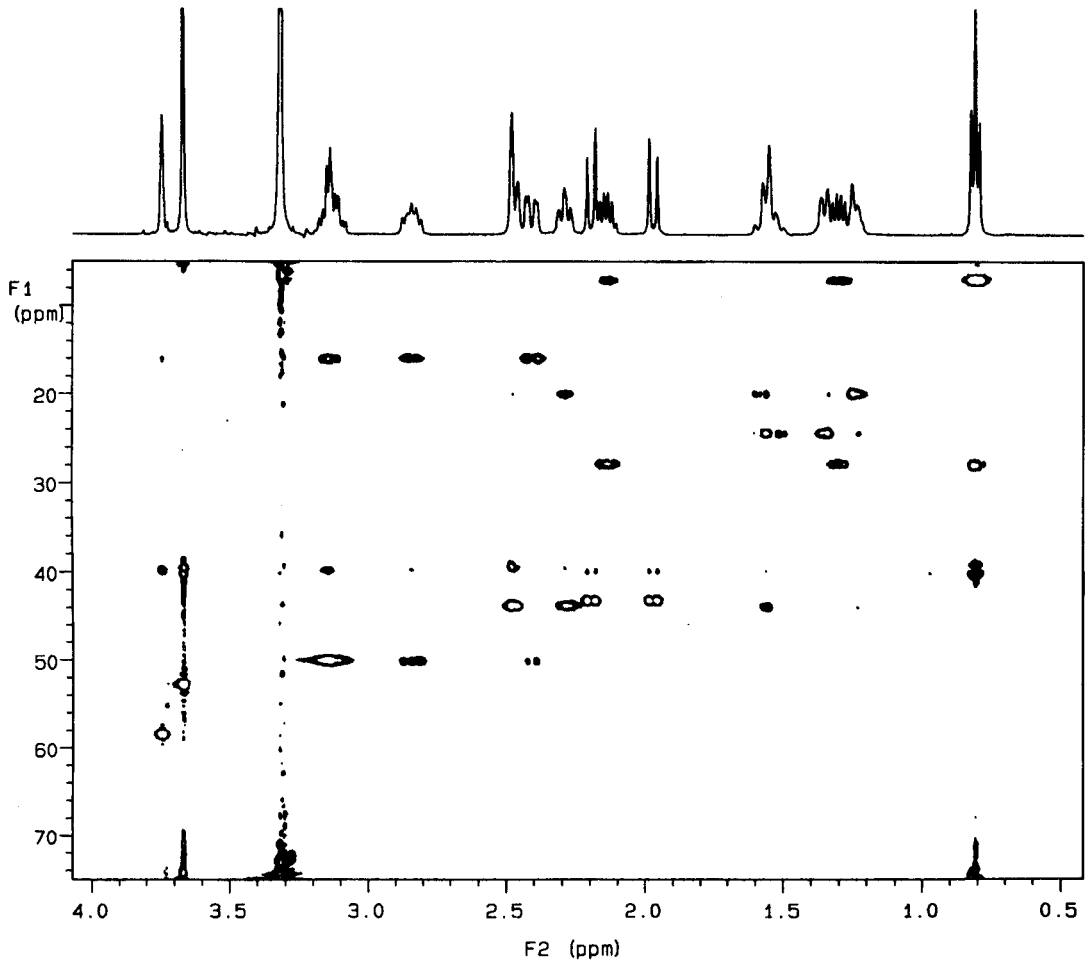
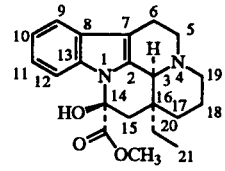


Figure 1. IDR-GHMQC-TOCSY spectrum of the aliphatic region of the alkaloid vincamine (1) in deuteriodimethyl sufloxide recorded with an 18 msec mixing time. Direct responses are phase inverted and are shown as red contours. Vicinally relayed responses have positive intensity and are denoted by black responses in the spectrum. The use of IDR-GHMQC-TOCSY obviates the acquisition of convention HMQC (or GHMQC) to establish direct proton-carbon correlations and either COSY or TOCSY experiments to establish proton-proton connectivities.

responses are plotted in red; vicinally relayed responses, which have positive intensity, are denoted by the black contours. The data were phased beginning from the direct response for the 21-Me group, which has obviously assignable proton and carbon chemical shift assignments. Likewise, the H3 singlet, resonating furthest downfield in the spectrum shown in Figure 1, also provided a good reference signal for first-order phasing. Following phasing, the methyl group is obviously coupled to the strongly anisochronous 20 methylene protons resonating at 2.15 and 1.30 ppm. Using the proton homonuclear connectivity information provided in the IDR-GHMQC-TOCSY spectrum, the H5a/e and H19a/e protons were first identified on the basis of chemical shift. They were readily differentiated from H3, which appeared as a singlet, and from the anisochronous 15a/e methylene protons which had no coupling other than their mutual geminal coupling. H5a/e and H19a/e were then differentiated from one another on the basis of the extent of their respective

Table I
Proton Resonance Assignments for the Alkaloid Vincamine (1) in d_6-DMSO at 500 MHz



Position	δ^1H	Position	δ^1H
H3	3.74	17a	1.56
H5a/e	3.12	17e	1.35
H6a	2.85	18a	1.53
H6e	2.41	18e	1.24
H9	7.38	19a	2.48
H10(a)	~7.00	19e	2.29
H11(a)	~7.00	20x	1.30
H12	7.03	20y	2.15
14-OH	6.78	21	0.79
15a	2.22	OMe	3.68
15e	2.11		

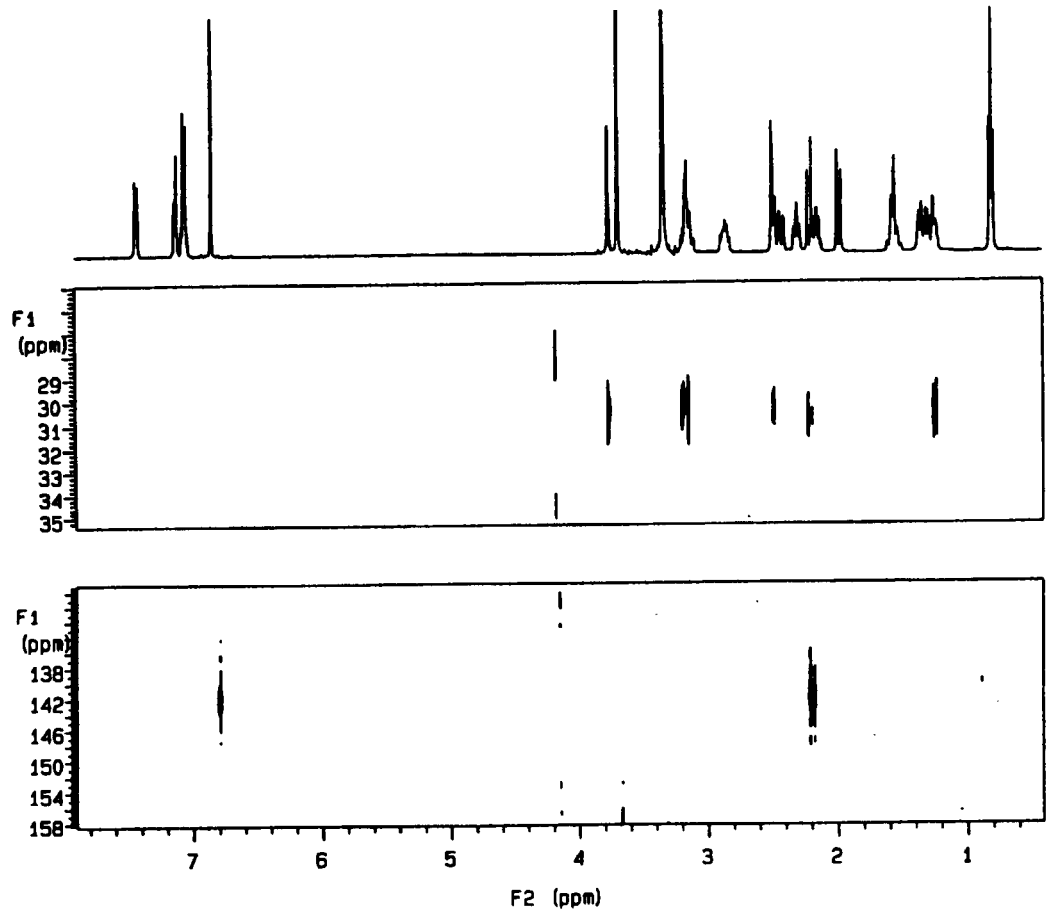


Figure 2. GHNMQC spectrum of the alkaloid vincamine (1) recorded with a 5 Hz optimization. The top panel shows correlations observed to the aliphatic N4 resonance at 31.5 ppm; the bottom panel shows correlations observed to the indole N1 resonance at 143.0 ppm.

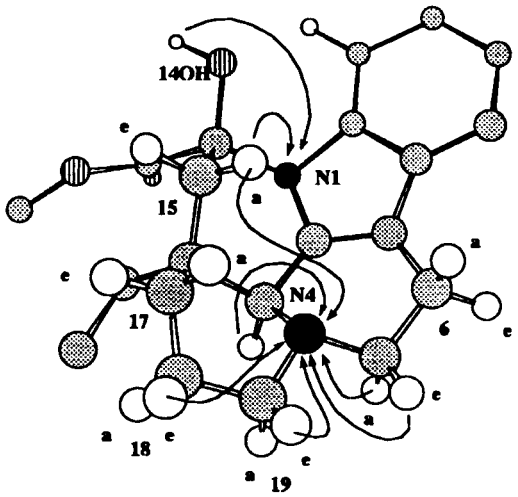
homonuclear coupling networks. The H5a/e protons were coupled only to the H6a/e protons. The H19a/e methylene protons were coupled first to 18a/e protons which were, in turn, coupled to the 17a/e protons. Resonance assignments for **1** in DMSO are presented in Table 1 and are consistent order-wise with those reported by other research groups. Furthermore, the assignments shown in Table 1 are also consistent in terms of their respective stereochemical assignations with the assignments of Moldvai [15] and co-workers.

With rigorous ^{1}H resonance assignments in hand, it was possible to undertake the acquisiton of the long-range ^{1}H-^{15}N heteronuclear chemical shift correlation spectrum of **1**. The data were recorded using the GHNMQC (Gradient-enhanced Hydrogen-Nitrogen Multiple Quantum Coherence) pulse sequence reported previously [1-5]; magnitude calculated spectral segments are presented in Figure 2. The upfield segment contains responses for the aliphatic N4 resonance; the downfield spectral segment contains the responses for the indole N1 resonance. The ^{15}N chemical shifts should be assumed to be accurate to approximately ±0.5 ppm.

The chemical shift of N1 was located at 143.0 ppm (downfield of liquid ammonia) on the basis of long-range responses to the 14-hydroxyl group and an intense long-range coupling to the 15a proton resonance at 2.22 ppm.

No response to the aromatic H12 proton resonating at 7.03 ppm was observed in the spectrum. A similar lack of three-bond couplings from the peri aromatic proton of indole systems has been noted for various members of the *Strychnos* alkaloid family [2] although it is by no means appropriate to state that this coupling should generally not be expected based on the relatively limited data thus far available. Rather, the observation of peri-aromatic proton to indole nitrogen couplings may be more generally dependant on the optimization of the GHNMQC experiment — *i.e.* the liklihood of observing a three-bond coupling such as H12-N1 in the present case would be greater when the experiment is optimized for smaller presumed long-range proton nitrogen couplings (*e.g.* 4-6 Hz).

Responses to the aliphatic N4 resonance at 31.5 ppm were more numerous. As expected, responses were observed to H3, to both of the H5 resonances, to H15a, and to the H19e and H18e protons. Initially, it was rather



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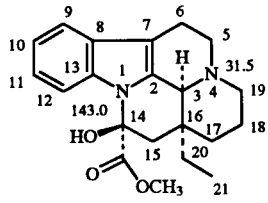
surprising to note that a long-range response was also observed to the H15a proton resonating at 2.22 ppm. Indeed, on initial inspection of the spectrum, this response might even be considered to be anomalous. From our experience in various alkaloid system two- and to a lesser extent three-bond couplings normally predominate. From the molecular model of the system, **3**, however, it is apparent that the H15a C-H bond vector and the N4 electron lone pair are synclinially oriented, which could account for the larger size and hence detectability of the $^4J_{H15a\text{-}N4}$ coupling. Similar augmentation in the size of couplings has been noted in the case of strongly synclinally oriented bond vectors relative to the nitrogen lone pair in the various *Strychnos* alkaloids we have previously studied [2]. In the case of the *Strychnos* alkaloids examined thus far, however, couplings augmented as a consequence of synclinal orientation have thus far always been two-bond ($^2J_{NH}$) correlations although there is no reason, *a priori*, that this phenomenon should be limited exclusively to two-bond coupling pathways.

Comparing vincamine (**1**) with the biogenetically related alkaloid ajmaline (**4**) [1] and the semi-synthetic bis indole alkaloid vinorelbine (**5**) [3] allows some general trends in chemical shift behavior to be noted. First, and not surprisingly, the indole and dihydroindole chemical shifts fall into distinctly different groups. The former tend to resonate around 140 ppm while the latter tend to cluster at about 70 ppm. Generalizations regarding the aliphatic nitrogen in the three alkaloids, in contrast, cannot be readily drawn. The N4 resonance of vincamine is observed further upfield than the corresponding resonances in either of the other two molecules. The N4 resonance of ajmaline (**4**) was observed at 53.0 ppm; the N9 and N5' resonances of vinorelbine (**5**) were observed at 55.3 and 44.0 ppm, in the velbanamine and vindoline portions of the molecule, respectively. Additional molecules will need to be studied before any empiricism can be developed for the behavior of the aliphatic ^{15}N chemical shift of *Vinca* alkaloids.



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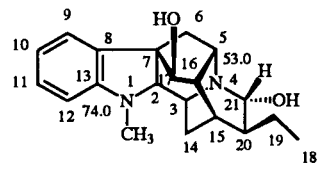

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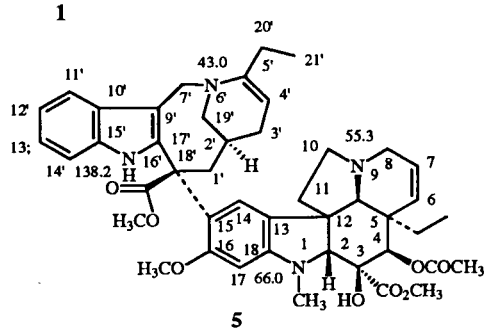

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EXPERIMENTAL

All spectra were acquired using a sample prepared by dissolving 15 mg of **1** in 550 µl d_6-DMSO in a standard 5 mm nmr tube (Wilmad). Experiments were performed on a Varian Unity 500 spectrometer equipped with three broadband rf channels, Performa II PFG hardware and a Nalorac Z•SPEC™ IDTG-500-5 gradient triple resonance inverse probe. Measured pulse widths for ^1H and ^{15}N were 9.8 and 30 µsec, respectively, at power settings of 56 and 63 dB, respectively (63 dB = maximum power). A maximum gradient strength of ~40 Gcm^{-1} (0.040 T) was possible with the combination of hardware used for the experiments described in this report.

The IDR-GHMQC-TOCSY spectrum shown in Figure 1 was acquired using the pulse sequence described by Crouch, Davis, and Martin [17] with a mixing time of 18 msec. Data were taken with gradient ratios of 2:2:2:1 and 2:2:2:-1 with gradient strengths of ~0.008T. The spectrum was acquired as 4096 x (64 x 2) hypercomplex files; data were zero-filled to 8192 x 256 points prior to processing. The data were taken using 8 transients/t_1 increment; no interleaving of the data was performed. The spectrum was acquired in approximately 2 hours.

The GHNMQC spectrum shown in Figure 2 was acquired as 4096 x (80 x 2) hypercomplex files using the pulse sequence previously described by Martin and Crouch [1]. Spectral widths in F_2 and F_1 were 3758 and 7092 Hz, respectively; the corresponding spectral regions were approximately 0.5-8.0 ppm for proton and 25-160 ppm for ^{15}N, respectively. The long-range delay in the experiment was optimized for 5 Hz. A total of 64 transients were accumulated/t_1 increment with a 1.5 sec interpulse delay giving a total acquisition time of 6 hours. The data were interleaved with 8 transients taken/t_1 increment pass. Gradient ratios used were 5:5:1 and 5:5:-1 [12] with a gradient strength of ~0.010 T. The data were zero-filled to 8192 x 512

points prior to processing, which used a gaussian multiplication prior to both Fourier transforms. Data presented in Figure 2 are shown as a magnitude calculation.

REFERENCES AND NOTES

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

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

[3] G. E. Martin and R. C. Crouch, *J. Heterocyclic Chem.*, **32**, 1839 (1995).

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

[5] K. A. Farley, G. S. Walker, and G. E. Martin, *Magn. Reson. Chem.*, submitted for publication (1997).

[6] A. Bax and S. Subramanian, *J. Magn. Reson.*, **67**, 565 (1986).

[7] A. Bax, R. H. Griffey, and B. L. Hawkins, *J. Magn. Reson.*, **55**, 301 (1983).

[8] A. Bax, R. H. Griffey, and B. L. Hawkins, *J. Am. Chem. Soc.*, **105**, 7188 (1983).

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

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

[11] J. Ruiz-Cabello, G. W. Vuister, C. T. W. Moonen, P. van Geldern, J. S. Cohen, and P. C. M. van Zilj, *J. Magn. Reson.*, **100**, 282 (1992).

[12] G. Kalaus, Z. Gyalai, M. Kajtar-Peredy, P. Gyoery, L. Szabo, and C. Szantay, *Acta Chim. Acad. Sci. Hung.*, **105**, 221 (1980).

[13] D. Genin, R. Z. Andriamialisoa, N. Langlois, and Y. Langlois, *J. Org. Chem.*, **52**, 353 (1987).

[14] A. Nemes, L. Czibula, G. Visky, M. Parkas, and J. Kreidl, *Heterocycles*, **32**, 2329 (1991).

[15] I. Moldvai, C. Szantay, Jr., K. Rissanen, and C. Szantay, *Tetrahedron*, **48**, 4999 (1992).

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

[17] R. C. Crouch, A. O. Davis, and G. E. Martin, *Magn. Reson. Chem.*, **33**, 889 (1995).