Long-Range ^{1}H-^{15}N Couplings and ^{15}N Chemical Shift Assignments of the Bis-Indole Anticancer Drug Navelbine®

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The recently reported application of gradient-enhanced HMQC for the observation of long-range ^{1}H-^{15}N heteronuclear couplings at natural abundance has been extended to the bis-indole anticancer drug Navelbine®. Responses correlating protons to N16' (δ ^{15}N 138.2 ppm)in the velbanamine subunit and N1 and N9 (δ ^{15}N 66.0 and 55.3 ppm, respectively) in the vindoline subunit were observable within about 4 hours of data acquisition. The single response to N6' (δ ^{15}N 43.0 ppm), in contrast, was very weak requiring a weekend data acquisition to even be observed. The difficulty in observing correlations to N6' is presumably a function of molecular mobility in the eight-membered azocine ring.

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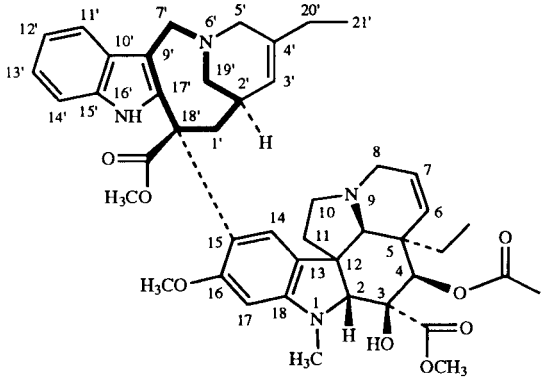
We recently reported an effective method of probing long-range $^{1}H^{15}N$ heteronuclear coupling pathways at natural abundance as a means of assigning ^{15}N chemical shifts of alkaloids [1] using ajmaline as a model compound for the study. Our initial study demonstrated that the orientation of a given hydrogen, synclinal or anticlinial to the nitrogen lone pair, had an effect on which couplings were observed and/or the intensity of the long-range coupling response, which is fully consistent with earlier reports on the behavior of long-range couplings to ^{15}N [2-5]. More recently, we extended our initial study to include three members of the *Strychnos* family of alkaloids [6]. We now wish to communicate the results of our initial studies of the long-range ^{1}H-^{15}N couplings with the much more complex bis-indole alkaloid anticancer drug Navelbine® (vinorelbine, **1**, 8'-noranhydrovinblastine).



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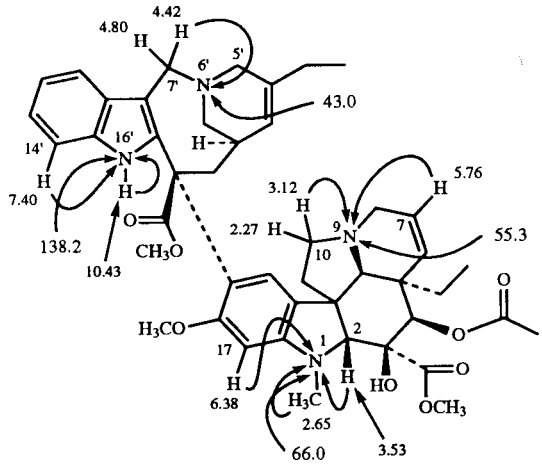
Rigorous proton resonance assignments for Navelbine (**1**) in d_6-DMSO were available form a previous study obviating the need for any reassignment [7]. From our previous studies [1,6] we expected the indole ^{15}N resonance contained in the velbanamine subunit of Navelbine to resonate furthest downfield, in the range of 130-150 ppm. In contrast, we expected the aliphatic nitrogen resonances to be in the range of ~40-70 ppm. Based on our recent study of the motional behavior of 3',4'-anhydrovinblastine [8], and the motional characteristics in the azanonine ring contained within holstiine [6], we anticipated that molecular motion in the eight-membered azocine (denoted by darkened bonds) in the velbanamine subunit of **1** could also experience sufficient motional freedom, making the observation of long-range ^{1}H-^{15}N couplings to N6' more difficult than the other three nitrogen atoms contained in the structure.

A sample consisting of 21 mg of Navelbine tartrate (1.9 mmoles) dissolved in 650 µl of 99.96% d_6-DMSO (Cambridge) was prepared for the long-range ^{1}H-^{15}N studies. A one-bond ^{1}H -^{15}N direct correlation experiment was performed first, the data acquired as 2048 x (16 x 2) hypercomplex files using a gradient-enhanced HMQC pulse sequence. A total of 24 transients/t_1 increment were accumulated giving a total acquisition time of 15 minutes. The one-bond ^{1}H-^{15}N coupling was optimized for 90 Hz; the spectral window used was from 120-150 ppm. From this spectrum (not shown) the N16' resonance in the velbanamine subunit was observed at 138.2 ppm *via* its one-bond correlation to the 16'-NH resonance at 10.43 ppm.

Following the acquisition of the ^{1}H-^{15}N direct correlation spectrum, a pair of long-range ^{1}H-^{15}N spectra were acquired using the sequence we described previously [1,6], the long-range delays optimized for 5 and 10 Hz. The spectra were acquired using a spectral window from 30-160 ppm as 4096 x (80 x 2) hypercomplex files. The data were zero-filled to 8K x 512 during processing.

Responses correlating protons to N16' in the velbanamine subunit and N1 and N9 in the vindoline subunit, as shown by **2**, were observable within about 4 hours of data acquisition in both experiments. Data acquisition in the 10 Hz optimized experiment was continued over a weekend, finally affording a spectrum (Figure 1) in which a single, weak long-range correlation from the proton resonating at 4.42 ppm could be observed to the N4' resonance at 43.0 ppm.

Individual traces from the 10 Hz optimized ^{1}H-^{15}N long-range correlation experiment are plotted in Figure 2 beneath a proton reference spectrum. These data likewise show the response correlating the H7' resonance to



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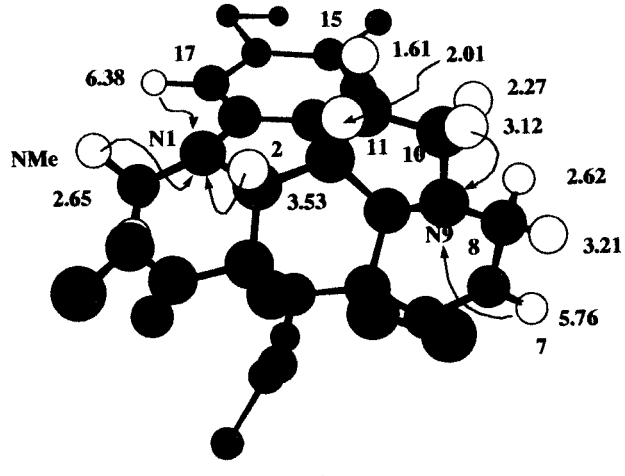
N6' to be the weakest response of any of the long-range correlations observed.

The molecular model of the vindoline subunit is shown by **3**. To improve the clarity of the presentation, the vindoline subunit was modeled with a methyl group at the 15-position *in lieu* of the velbanamine subunit, the latter having no impact on the conformation in the vindoline subunit when the full structure was modeled. We have employed a similar approach in our modeling work with 3',4'-anhydrovinblastine [8]. Long-range ^{1}H-^{15}N couplings to N1 and N9 are shown. The coupling from the NMe group into N1, as noted from the trace in Figure 2, is considerably weaker than the response from the peri H17 resonance. In our previous experience [1,6] three-bond peri couplings into ^{15}N were generally quite weak and difficult to observe, making the intensity of the peri coupling in this case somewhat paradoxical. The intensity of the two-bond coupling of H2 to N1, since H2 is synclinal to N1, is quite reasonable [2-5].

Long-range couplings into N9 are also shown by **3**. Two couplings were observed, a two-bond coupling from the H10 proton resonating at 3.12 ppm and a three-bond coupling from the H7 vinyl proton. The stereochemical assignments of the protons of the 10/11 ethylene bridge were established from a ROESY experiment. The two-bond coupling from H10 is again reasonable since this proton is oriented synclinally to the N9 lone pair. Why, however, no two-bond coupling was observed to the corresponding H8 proton resonating at 3.21 ppm is by no means obvious.



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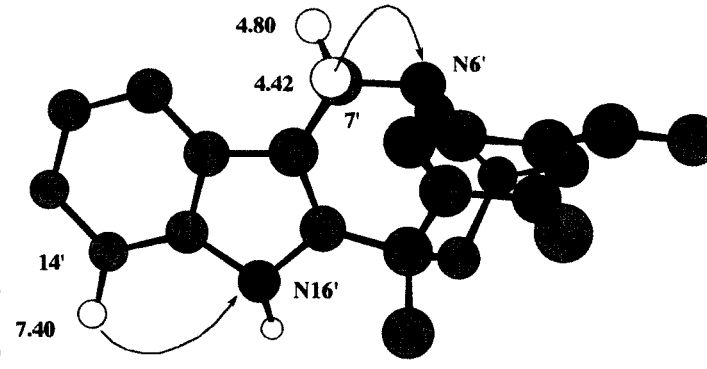
The three-bond ^{1}H-^{15}N coupling between H7 and N9 in the vindoline unit is also quite reasonable given that the dihedral angle between H7 and N9 which defines this coupling pathway is -176.9°. Three-bond long-range ^{1}H-^{15}N couplings obey a Karplus relationship which has been shown to have maxima near 0° and 180° [2-5].

The molecular model of the velbanamine subunit is shown by **4**; the molecule was modeled with the vindoline subunit replaced by a methyl group to improve clarity. The stereochemical orientation of the 7' protons was again established from a ROESY spectrum. The sole long-range ^{1}H-^{15}N coupling to N6' was observed from H7' resonating at 4.42 ppm oriented nearly orthogonally to the N6' lone pair of electrons, which would hence be expected to result in a weak coupling. Again, it is not obvious why neither of the H5' resonances are coupled to N6'.



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Finally, aside from it's one-bond direct correlation, N16' is also again strongly coupled to the H14' peri proton. As noted above, the result is contrary to the general behavior we have observed for this coupling pathway in the *Strychnos* alkaloids [6] although it is also important to note that the corresponding peri coupling in the *Strychnos* alkaloids is to an amide nitrogen.

In conclusion, long-range ^{1}H-^{15}N heteronuclear shift correlation at natural abundance has allowed the four nitrogen atoms of the complex bis-indole anticancer alkaloid Navelbine to be successfully assigned. Three of the four ^{15}N resonances were readily observed in a few hours, the fourth, N6', because of the stereochemical disposition of protons at the vicinal 5' and 7' positions, had only a very weak coupling pathway which was far more difficult to observe, requiring data acquisition over a weekend. As

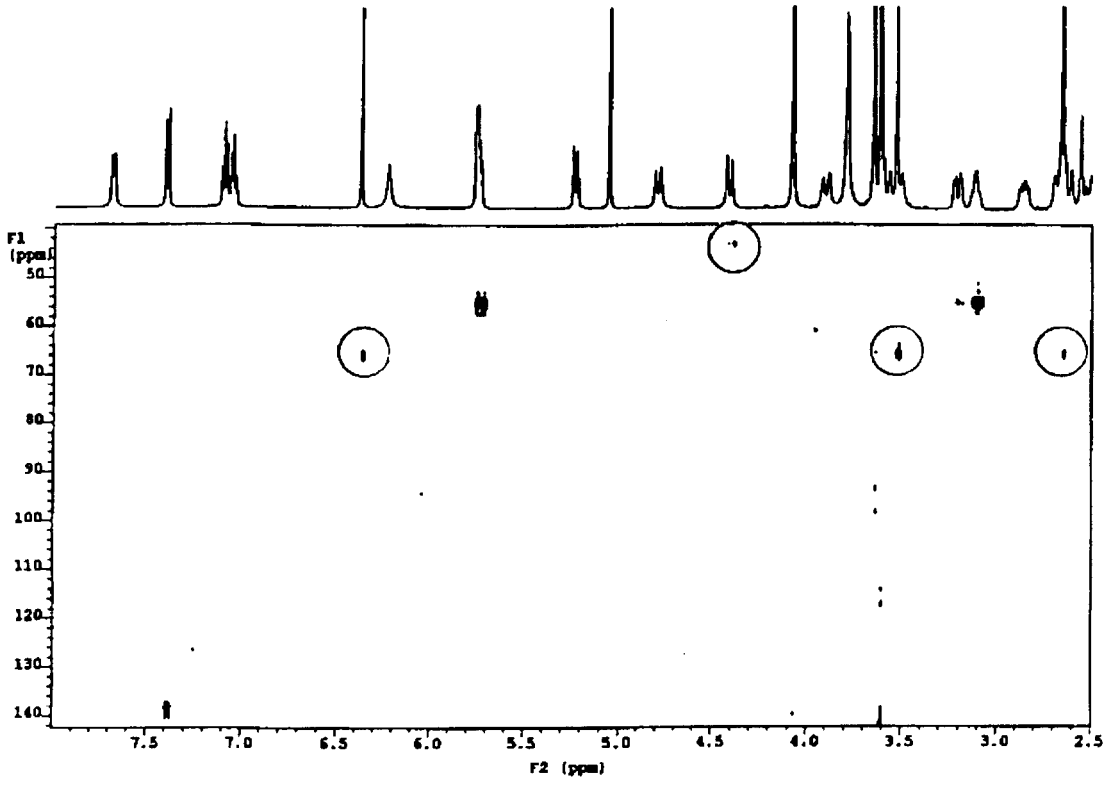

Figure 1. Long-range inverse-detected natural abundance ^{1}H-^{15}N gradient HMQC spectrum [1] of a solution of 1.9 mmoles of Navelbine in 650 µl Id_6-DMSO recorded at 500 MHz. The experiment was optimized for a long-range coupling of 10 Hz. The data were acquired as 4096 x (80 x 2) hypercomplex files over a weekend.

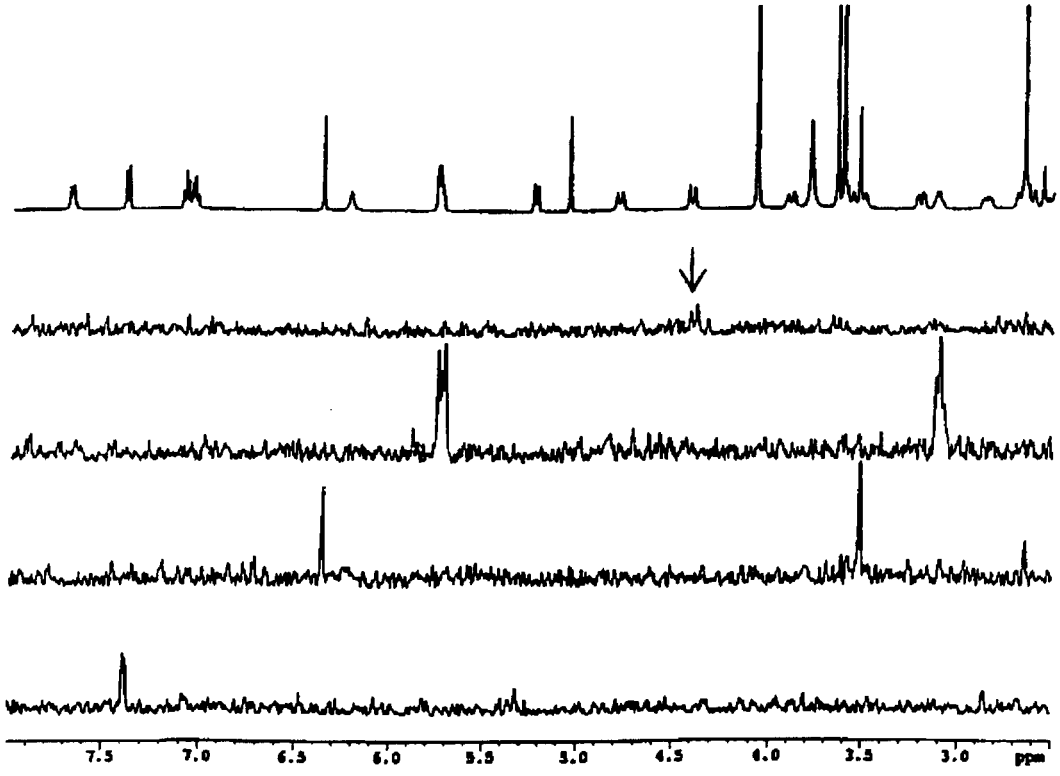

Figure 2. Slices taken from the 10 Hz optimized long-range natural abundance ^{1}H-^{15}N HMQC spectrum shown in Figure 1. Plotted from top to bottom: reference spectrum (top); responses to the N6' resonance at 43.0 ppm; responses to the N9 resonance at 55.3 ppm; responses to the N1 resonance at 66.0 ppm; responses to the indole N16' resonance at 138.2 ppm (bottom).

observed previously in the *Strychnos* alkaloids [6], the orientation of protons capable of coupling to a given nitrogen relative to the lone-pair of electrons of that nitrogen plays a key role in the intensity of the responses observed.

References and Notes

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