Long-Range ^1H-^{15}N Correlation at Natural Abundance Using Gradient-Enhanced Inverse-Detection

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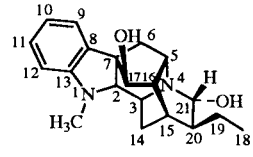
Received June 28, 1995

Inverse-detected heteronuclear shift correlation efficiency has been significantly augmented by the incorporation of pulsed field gradients (PFG). Phase-cycling requirements for t_1-noise suppression in gradient-enhanced experiments are, for the most part, obviated, making it feasible to acquire data in one or a few transients/t_1 increment. The benefits which accrue for ^1H-^{13}C correlation (using GHMQC, GHMBC, and variants of GHMQC-TOCSY) are well documented. Less obvious is the increased facility with which long-range ^1H-^{15}N correlation spectra can be acquired. An IDR-(Inverted Direct Response)-GHMQC-TOCSY was used to establish unequivocal proton resonance assignments for the alkaloid ajmaline. Long-range ^1H-^{15}N heteronuclear couplings to the two nitrogen atoms of ajmaline were then probed using a gradient-enhanced ^1H-^{15}N heteronuclear shift correlation experiment derived from HMQC. Long-range ^1H-^{15}N couplings in ajmaline are assigned for the first time.

J. Heterocyclic Chem., **32**, 1665 (1995).

Two-dimensional nmr techniques have had an inarguably profound impact on the way in which structure elucidation and spectral assignments studies are conducted. Heteronuclear shift correlation experiments, in particular, have undergone several rounds of modification. The initial HETCOR [1] and long-range optimized heteronucleus-detected experiments [2] have been almost uniformly supplanted by ^1H- or inverse-detected variants. Direct or one-bond correlation experiments are now performed using HMQC [3] or HSQC [4] in most laboratories. Long-range correlations are nominally established using the HMBC experiment pioneered by Bax and Summers [5]. Homonuclear proton-proton connectivities in heavily congested spectra can be readily untangled using HMQC-TOCSY [6] or a phase-edited variant such as IDR-(Inverted Direct Response)-HMQC-TOCSY [7,8]. The latter technique, employed in concert with HMQC and HMBC experiments using a micro inverse-detection probe [9,10], were, for example, crucial in the total assignment of the ^1H- and ^{13}C-nmr spectra of the marine toxin brevetoxin-3 [11].

Further improvement of the inverse-detected experiments was afforded by the introduction of pulsed field gradients. The gradient-enhanced variants of the HMQC and HMQC-TOCSY experiments were described by Hurd and John [12] and John *et al* [13], respectively. An excellent treatment of the fundamentals of gradient-enhanced inverse-detected experiments are presented in the work of van Zilj and co-workers [14]. More recently, we have described a phase-sensitive GHMQC-TOCSY experiment with direct response editing capabilities [15]. We now illustrate the utilization of IDR-GHMQC-TOCSY, which afforded a quick and convenient means of unequivocally assigning the proton resonances of the alkaloid ajmaline (**1**) at 500 MHz necessary to support the study of the long-range ^1H-^{15}N connectivities of **1** at natural abundance.



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All of the spectra described in this report were acquired using a sample of 19.1 mg (0.058 mmole) of ajmaline (**1**) dissolved in 650 µl 99.96%D d_6-DMSO (CIL). The data presented were acquired using a three channel Varian Unity™ 500 spectrometer equipped with Performa II PFG hardware and a Nalorac IDTG-500-5 Z•SPEC® 5mm triple resonance (^1H, ^{13}C, ^{15}N) probe. Measured 90° pulse widths were 9.7, 11.0, and 30.0 µsec at power settings of 56, 61, and 63 dB (63 dB maximum) for ^1H, ^{13}C, and ^{15}N, respectively. A maximum gradient strength of ~40 Gcm^{-1} (0.040 T) was possible with the combination of hardware used.

Direct proton-carbon correlations and proton-proton connectivities were established from an IDR-(Inverted Direct Response)-GHMQC-TOCSY spectrum acquired using the pulse sequence shown in Figure 1 giving the spectrum shown in Figure 2 with a mixing time of 18 msec. Phasing was established using previously reported proton/carbon resonance assignments [16], the *N*-methyl group providing an internal frame of reference for accurate phasing. Data were taken with gradient pairs of 2:2:2:1 and 2:2:-2:-1; DAC values of 6000:6004:6035:3020 for gt1-gt4, where 6000 = 0.008T, were employed.

Before considering the long-range ^1H-^{15}N correlation experiments performed on ajmaline (**1**), it is beneficial to briefly reconsider the historical development of inverse-detected ^1H-^{15}N spectroscopy and applications of ^{15}N-nmr spectroscopy in alkaloid chemistry. Following the initial pioneering development of heteronuclear multiple

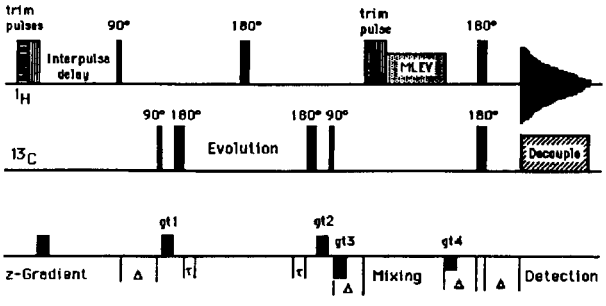

Figure 1. Pulse sequence used for IDR-(Inverted Direct Response)-GHMQC-TOCSY [15]. The fixed delays, Δ, were optimized as a function of $1/2(^1J_{CH})$ giving 3.6 msec where J = 140 Hz; the fixed delays, τ, were set equal to the duration of the gradient times which were uniformly 1.5 msec. In the case of delays containing a gradient (gt3 and gt4) the total duration of the delay was held constant at 3.6 msec with the gradient applied during the first 1.5 msec. The experiment was performed with gradient ratio pairs of 2:2:2:1 and 2:2:-2:-1; DAC values of 6000:6004:6035:3020 for gt1-gt4, where 6000 = 0.008T, were employed. The gradient following the proton trim pulses was utilized as a homospoil with the power and duration set equivalent to gt1. The 180° ^{1}H/^{13}C pulse sandwich following the MLEV isotropic mixing interval was used to selectively invert direct responses. Elimination of this pulse sequence element would give a conventional phase-sensitive GHMQC-TOCSY spectrum with direct and relayed responses of identical phase. Employing a 90° ^{13}C pulse in lieu of the final ^{13}C 180° pulse would afford a spectrum in which direct responses are suppressed obviating the need for broadband decoupling during acquisition and allowing, if necessary, higher levels of digital resolution to be employed in F_2 than would otherwise be possible.

quantum coherence by Müller [17], ^{1}H-^{15}N heteronuclear shift correlation *via* multiple quantum coherence was described by Bax and co-workers in 1983 [18,19] several years prior to the development of the HMQC sequence [3] now in widespread usage for ^{1}H-^{13}C heteronuclear shift correlation. The evolution of inverse-detected nmr techniques through the incorporation of gradients [12-14] is thoroughly treated in the recent chapter by Griesinger and co-workers [20] to which the interested reader is referred for further details.

Having set the stage experimentally for ^{1}H-^{15}N long-range heteronuclear shift correlation it is germane to consider what is contained in the literature on the ^{15}N chemical shift properties of alkaloids. Unfortunately, because of the low natural abundance and gyromagnetic ratio of ^{15}N, there is a dearth of information on the ^{15}N shifts of alkaloids in general. The earliest paper of which we are aware was the direct ^{15}N observation study reported by Fanso-Free and co-workers [21] of several quinolizidine alkaloids including reserpine and yohimbine. After a gap of eleven years, the next paper reporting ^{15}N data on an alkaloid was that of Carmeli and co-workers [22] which contained ^{15}N shift assignments for an oxazole/thiazole-derived alkaloid named didehydrotantazole-A. While this work utilized inverse-detection, it should be noted that the sample studied was >90% ^{15}N enriched from label feeding experiments. In 1993, we reported partial ^{15}N data for

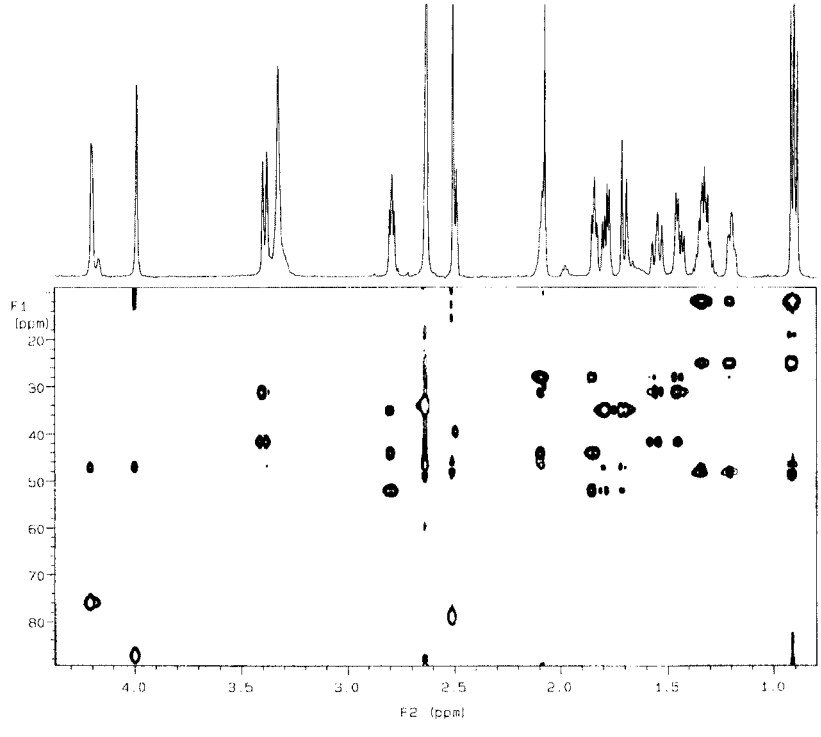

Figure 2. IDR-GHMQC-TOCSY spectrum of ajmaline (**1**) recorded with an 18 msec mixing time.

the protonated nitrogen of the complex spiro nonacyclic alkaloid cryptospirolepine [23]. The first experimental effort to probe long-range ^1H-^{15}N correlations of indoloquinoline alkaloids at natural abundance was contained in a poster presented by the authors in 1993 [24]. Finally, we have recently reported [25] the ^{15}N shifts of the minor alkaloid quindolinone using one-bond ^1H-^{15}N HMQC micro inverse-detection on a sample consisting of 3.4 μmoles.

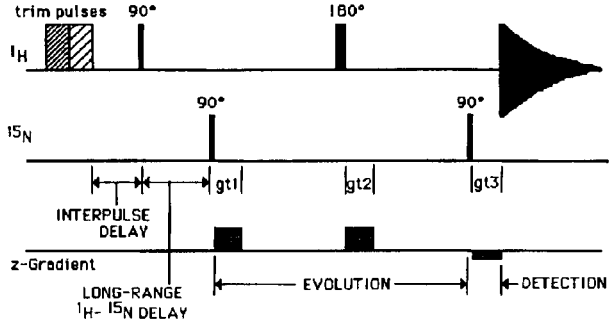

Figure 3. Gradient-enhanced HMQC-derived sequence used for long-range ^1H-^{15}N heteronuclear shift correlation. Pulsed field gradients gt1-gt3 had uniform durations of 1.5 msec; gradient pairs of 5:5:1 and 5:5:-1 were used with tweaked DAC values of 10000, 10000, 2010. The long-range delay was variously optimized for 5, 8, 10, and 12 Hz.

With the number and, in some cases, medical importance of alkaloids or their semi-synthetic derivatives, it is appropriate to begin to study the long-range ^1H-^{15}N correlation behavior with the ultimate aim of being able to use this information to augment structure elucidation and/or stereochemical inferences whenever necessary and feasible. The long-range ^1H-^{15}N heteronuclear correlation

Table 1

Proton Chemical Shifts, Homonuclear Couplings, Long-Range ^1H-^{15}N Coupling Pathways, ^{15}N Shifts, and ^1H-^{15}N Heteronuclear Couplings of Ajmaline (1)

Position	δ^1H	Homonuclear J Coupling (Hz) and Multiplicity	$\delta^{15}N$ [a]	Long-Range ^1H-^{15}N Correlations [b] and Couplings (Hz) [c]
N1			74.0	H2, H3
H2	2.505	singlet		$^3J_{N4H2} = 4$ $^2J_{N1H2} = 3.5$
H3	3.389	d, J = 10		$^2J_{N4H3} = 3.5$ $^3J_{N1H3} = 3.5$
N4			53.0	H2, H3, H5, H6a/e, H21
H5	2.790	dd, J = 5.9		$^2J_{N4H5}$ [d]
H6a	1.787	dd, J = 5.6, 11.7		$^3J_{N4H6a}$ [d]
H6e	1.700	d, J = 11.7		$^3J_{N46e} = 3.5$
H9	7.410	dd, J = 7.3, 1.3		
H10	6.651	ddd, J = 7.5, 7.5, 1.2		
H11	7.010	ddd, J = 7.8, 7.5, 1.5		
H12	6.601	broad d, J = 7.8		
H14a	1.547	dd, J = 12.9, 10.4		
H14e	1.441	dd, J = 12.9, 5.3		
H15	2.086	multiplet		
H16	1.840	multiplet		
H17	4.203	singlet		
18Me	0.903	t, J = 7.2		
19 CH_2	1.330	multiplet		
20	1.197	dddd, J = 11.3, 7.9, 3.2, 1.2		
21	3.988	singlet		$^2J_{N4H21} = 6.5$
NMe	2.627	singlet		

[a] Nitrogen chemical shifts are reported in ppm downfield from the position of liquid ammonia at 50.65 MHz. Nitrogen shifts can be rereferenced to neat nitromethane which resonates 379.5 ppm downfield of liquid ammonia. [b] All protons coupled to a given nitrogen are shown for the nitrogen position. [c] Long-range ^1H-^{15}N couplings are rounded to the nearest 0.5 Hz and are estimated to be accurate to ±1 Hz. [d] The coupling could not be extracted from the phased F_2 trace from the 2D spectrum.

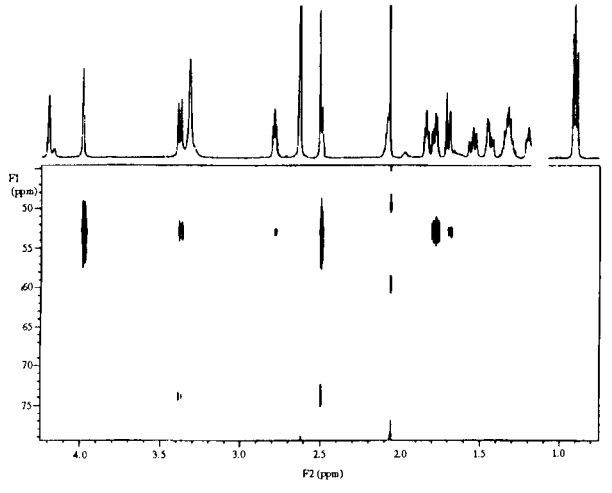

Figure 4. Long-range gradient-enhanced ^1H-^{15}N heteronuclear shift correlation spectrum of ajmaline (1).

experiments were performed using the gradient-enhanced pulse sequence shown in Figure 3. The experiment shown is essentially the same experiment as that we have previously described [24] except for the inclusion of gradients in the present work. The delay for long-range ^1H-^{15}N coupling between the first ^1H and ^{15}N pulses was variously optimized for 5, 8, 12, and 10 Hz. All data were acquired as 4096 x (70 x 2) hypercomplex files giving acquisition times of 0.537 and 0.009 sec in t_2 and t_1, respectively. The F_1 spectral window utilized was 10-80 ppm for ^{15}N. A total of 64 transients/t_1 increment was accumulated for each of the first three experiments giving total acquisition times of 4.5 hours; the 10 Hz experiment was acquired in an open-ended fashion. A total of 128 transients/t_1 increment was accumulated for this experiment giving a total acquisition time of 9 hours. The latter spectrum is shown in Figure 4 and differed only in that the signal-to-noise for the weaker responses was some-

what better. The same set of responses was qualitatively observed for both nitrogens in all of the experiments performed though there were some quantitative differences in response intensity as a function of optimization.

Responses were observed in the long-range ^{1}H-^{15}N spectrum for two nitrogens, one resonating at 53.0 ppm, the second at 74.0 ppm. As is clearly evident from the spectrum shown, responses to the former were both more numerous and significantly more intense. Referring to the proton resonance assignments (Table 1), responses to the ^{15}N resonance at 53.0 ppm were observed from H21, H3, H5 (weak), H2, H6a, and H6e (weak). There were only two responses to the 74.0 ppm ^{15}N resonance, a weak response from H3 and a somewhat more intense response from H2. Given the correlations observed, the nitrogen resonating at 53.0 ppm is readily assigned as N4; the nitrogen resonance at 74.0 ppm is assignable as the methylated N1 resonance of the dihydroindole nucleus.

Using the ChemDraw/Chem3D programs, the structure of ajmaline (**1**) shown in Figure 5 was modeled and minimized. Aside from the hydroxyl hydrogens, only the hydrogen atoms coupled to one or both of the ^{15}N resonances are shown. Of the couplings to the N4 resonance, three are *via* $^{2}J_{NH}$ and three are *via* $^{3}J_{NH}$. The $^{2}J_{NH}$ correlation from H21 and the $^{3}J_{NH}$ coupling to H2 are comparable and the most intense responses in the spectrum (see traces shown in Figure 6).

Relative to the response between N4-H21, the two-bond coupling from H3-N4 is weaker and that from H5-N4 is very weak. If we examine the minimized model (Figure 5), we observe that H21 and the N4 lone pair are in closest proximity while the H3 and H5 are further removed from the N4 lone pair. These orientations are reasonably consistent with the greater intensity of N4-H21 correlation response since $^{2}J_{NH}$ couplings depend strongly on the presence or absence of a nitrogen lone pair and the orientation of the proton in question synclinally or anticlinally to the lone pair [26-30].

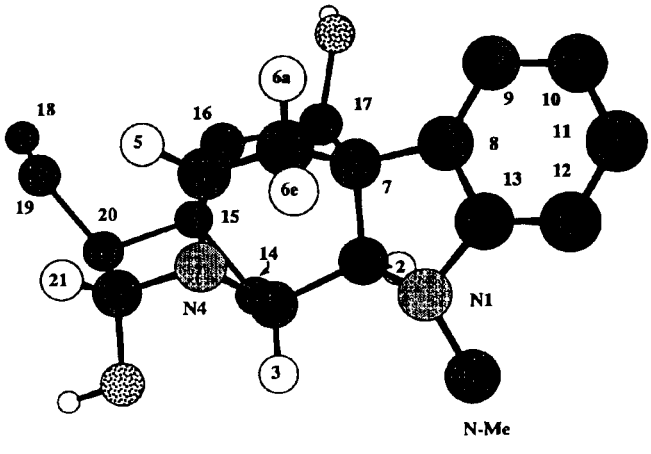

Figure 5. Minimized structure of ajmaline (**1**) showing hydrogens long-range coupled to N1 and N4. Dihedral angles for the $^{3}J_{NH}$ coupling pathways were: -24.9° for N1-H3; -147.3° for N4-H2; -162.1° for N4-H6a; and -36.7° for N4-H6e.

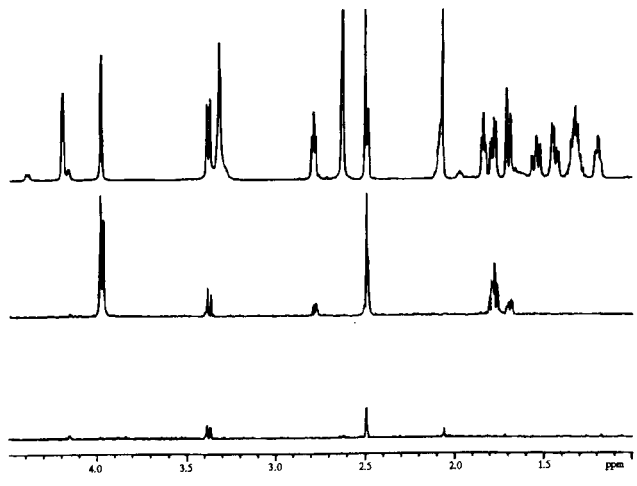

Figure 6. Slices taken at 53.0 (middle) and 74.0 ppm (bottom) from the long-range ^{1}H-^{15}H heteronuclear shift correlation spectrum of ajmaline (**1**) shown in Figure 4. The proton reference spectrum of ajmaline is shown as the top trace.

The relative intensities of the $^{3}J_{NH}$ correlations from H6a (1.787 ppm) and H63 (1.700 ppm) to N4 are quite interesting. From Figure 5 the dihedral angle formed between H6a and N4 is -162.1° while in contrast the dihedral angle between H6e and N4 is -36.7°. For comparison, the dihedral angle between H2-N4 was -147.3°. A $^{3}J(^{15}N$-C-C-$^{1}H)$ Karplus-type relationship has been shown to govern the magnitude of $^{3}J_{NH}$ couplings [27] with maxima at or near 0° and 180° and a minimum in the vicinity of 90°. On this basis, it is to be expected that the $^{3}J_{NH}$ couplings from H6a and H2 to N4, with dihedral angles of -162.3° and -147.3°, respectively should be larger than that of H6e-N4 with a dihedral angle of -36.7°. Given that the data shown in Figure 4 are from a 10 Hz optimized long-range ^{1}H-^{15}N heteronuclear correlation experiment, the greater intensity of the H6a-N4 and H2-N4 correlations would be consistent with long-range ^{1}H-^{15}N couplings that are better accommodated in the 10 Hz optimization. Extrapolating further, it is attractive to speculate that the apparent dihedral angle dependence observed in this case is general and can be used, *a priori*, to predict which of a pair of anisochronous methylenes will couple to a given nitrogen without having to make unequivocal proton resonance stereochemical assignments in every case.

In conclusion, the ability to observe ^{1}H-^{15}N long-range couplings is greatly facilitated using the gradient-enhanced pulse sequence shown in Figure 3 relative to a conventional non-gradient experiment [24]. While a rigor-

ous comparison of gradient and non-gradient techniques remains to be done, the freedom from t_1 noise inherent to the gradient experiment should translate to an improvement in the ability to perform the experiment on a given sample by perhaps as much as an order of magnitude. Conversely, the gradient technique should also make it possible to study long-range ^1H-^{15}N coupling pathways in smaller samples than would be feasible with a non-gradient experiment, particularly when used with a gradient micro inverse probe. Finally, more work needs to be done to evaluate potential modulation effects in the long-range ^1H-^{15}N experiment, which are presumably a sin modulation dependent on the magnitude of the long-range $^nJ_{NH}$ coupling.

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