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Determination of ^{15}N Chemical Shifts and Remote Connectivities in Selected Alkaloids by Gradient-Enhanced Inverse-Detected NMR Experiments

Radek Marek[1]*, Jirí Dostál[2], Jirí Slavík[2] and Vladimír Sklenár[1]*

[1]NMR Laboratory, Faculty of Science, Masaryk University, Kotlárská 2, CZ-611 37 Brno, Czech Republic, Phone: +42-5-41129383, Fax: +42-5-41211214 (rmarek@chemi.muni.cz, sklenar@chemi.muni.cz)

[2]Department of Biochemistry, Faculty of Medicine, Masaryk University, Komenského nám. 2, CZ-662 43 Brno, Czech Republic (jrdostal@med.muni.cz)

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Abstract

Determination of ^{15}N chemical shifts and long range connectivities of the alkaloids roemeridine, armepavine, chelerythrine and sanguilutine is presented. In addition, the complete assignment of ^{1}H- and ^{13}C-NMR chemical shifts of roemridine is reported.

Keywords: Armepavine, chelerythrine, roemeridine, sanguilutine, inverse-detected NMR, ^{15}N chemical shifts

The NMR experiments correlating the proton and nitrogen (^{15}N) chemical shifts across several bonds supply valuable information for molecular structure determination of organic compounds. The original experiments relying on the direct observation of ^{15}N nuclei have been outdated by *proton*- or so called *inverse*-detected variants with substantially higher sensitivity [1]. Recently, significant improvement of the inverse-detected experiments has been attained by the introduction of pulsed field gradients (PFG) [1]. The gradients have been implemented to eliminate the t_1 noise, improve the solvent suppression and remove the spectral artifacts.

The ^{15}N chemical shifts and long range coupling pathways can be detected by gradient-enhanced HMBC [2] and HSQC [3] experiments. In addition, the measurements of long range coupling constants is greatly facilitated by a phase-sensitive gradient-enhanced HSQC experiment optimized to detect remote connectivities [4].

Here we present initial results obtained by application of gradient-enhanced experiments for the measurement of ^{15}N chemical shifts, detection of remote connectivities and assesment of ^{1}H-^{15}N long range coupling constants in alkaloids roemeridine (**1**) [5], armepavine (**2**) [6, 7], chelerythrine (**3**) [8-10] and sanguilutine (**4**) [8-10].

* *To whom correspondence should be addressed*

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*Roemeridine (**1**)*

A sample of 52 mg of roemeridine (**1**) was dissolved in 500 µl of d_6-DMSO. The signals in the ^{1}H-NMR spectrum were assigned using gradient-enhanced DQF- and TQF-COSY [11]. Complete assignments of ^{13}C resonances was obtained from 2D ^{1}H–^{13}C-HSQC and -HMBC spectra (see Experimental Section). The ^{1}H-^{15}N-HMBC experiment optimized to detect remote connectivities with scalar coupling constants of approximately 7 Hz showed a clear interaction of H(N2) with its directly bonded nitrogen N2. The same nitrogen is coupled with hydrogen atom H19 (7.07 ppm). Responses arising from interactions of the nitrogen N1 with protons two or three bonds away were also observed in the same experiment. Interactions of the nitrogen atom N3 with neighbouring hydrogens were detected only in experiment with the evolution delay set to observe long range interactions amounting to 4 Hz. All measurable ^{1}H–^{15}N interactions are summarized in Fig. 1.

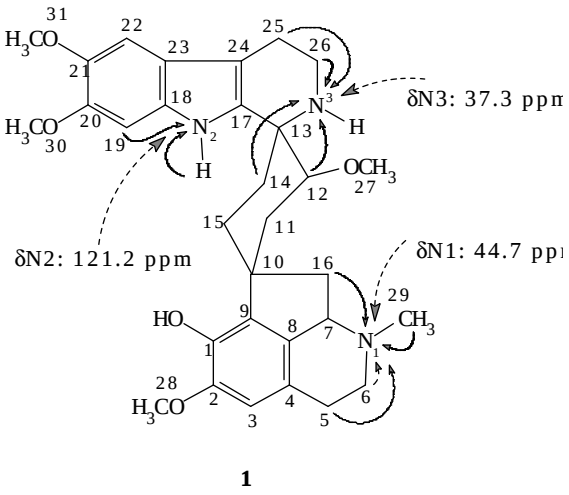

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Figure 1. *The ^{15}N chemical shifts and long range ^{1}H-^{15}N interactions observed for alkaloid roemeridine (**1**)*

The gradient-enhanced phase-sensitive ^{1}H-^{15}N-HSQC experiment enabled us to estimate the values of long range coupling constants from the antiphase patterns [12]. For the three-bond scalar interaction of H19 to N2 the value of ^{3}J(H19, N2) = 5.6 Hz was obtained. The coupling constant for the two-bond interaction between methyl protons H29 and N1 was also measured (^{2}J(H29, N1) = 5.3 Hz). From the ^{1}H-^{15}N-HSQC spectra the relative sign of the coupling constants can be determined. Considering the negative sign of a single-bond proton-nitrogen interaction (^{1}J(H2,N2) = -99 Hz) all the coupling constants discussed above must be positive since antiphase doublets of long range interactions showed inverted patterns.

*Armepavine (**2**)*

An NMR sample was prepared by dissolving 30 mg of armepavine (**2**) in 500 µl of $CDCl_3$. The signals in ^{1}H- and ^{13}C-NMR were assigned applying the same strategy as in the case of roemeridine (**1**). The most intense correlation in the ^{1}H-^{15}N-HMBC spectrum was observed for the interaction of nitrogen atom N2 with the methyl group hydrogens H-12. The ^{1}H-^{15}N-HMBC experiment allowed us to trace the remote connectivities in armepavine (**2**), but did not permit to extract the values of small heteronuclear coupling constants. All the observed ^{1}H-^{15}N multiple bond connectivities are shown in Fig. 2.

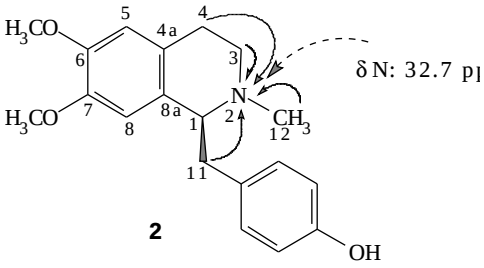

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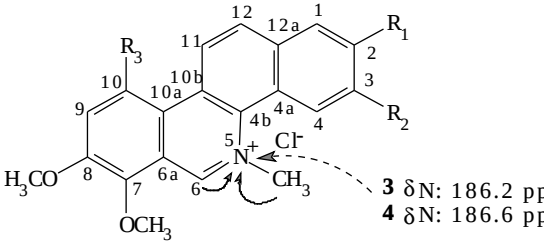
Figure 2. *The ^{15}N chemical shift and long range ^{1}H-^{15}N interactions observed for alkaloid armepavine (**2**)*

*Chelerythrine chloride (**3**) and Sanguilutine chloride (**4**)*

Chelerythrine (**3**) and sanguilutine (**4**) are biogenetically related benzo[c]phenanthridine alkaloids [8]. The samples were prepared by dissolving the alkaloids (10-15 mg) in 500 µl of d_6-DMSO. Assignment of ^{1}H resonances of chelerythrine and sanguilutine was described previously [8]. The only interaction observed in ^{1}H-^{15}N-HMBC and HSQC experiments is a correlation of N5 with H6 and with the hydrogen atoms of the directly bonded methyl group. Two-bond coupling constants were measured from the HSQC spectra: the same values ^{2}J(H6,N5) = 4.7 Hz and ^{2}J(CH_3,N5) = 4.4 Hz were obtained for compounds **3** and **4**. The ^{15}N chemical shifts, ^{1}H-^{15}N coupling pathways and atom numbering are shown in Fig. 3.

The results presented for alkaloids armepavine, chelerythrine, roemeridine, sanguilutine clearly demonstrate the power of gradient-enhanced, inverse-detected NMR experiments for ^{15}N and ^{13}C chemical shift assignment and measurements of long-range coupling constants. The recently developed experiments provide routine access to parameters previously obtainable only with difficulty.



3: $R_1 + R_2$ = O-CH_2-O, R_3 = H
4: $R_1 = R_2 = R_3 = OCH_3$

Figure 3. *The ^{15}N chemical shifts and long range ^{1}H-^{15}N interactions observed for alkaloids chelerythrine (**3**) and sanguilutine (**4**).*

Experimental Section

General Details

NMR spectra were measured on a 500 MHz Bruker DRX Avance spectrometer, δ values are in ppm, coupling constants in Hz. The experiments were carried out using the 5 mm triple-resonance probehead ($^{1}H\{^{13}C/BB\}$) equipped with a z-gradient coil using the following sequences:

^{1}H-^{15}N HMBC: D1-90°(^{1}H)-D6-90°(^{15}N)-t_1/2-GP1-D16-180°(^{1}H)-GP2-D16-t_1/2-90°(^{15}N)-GP3-D16-ACQ(t_2); acquisition parameters: D1 = 2.3 s, D6 = 60-120 ms, D16 = 100 μs, GP = 1 ms, 16-128 scans were acquired per t_1-increment, block size 4096×512 points, gradient amplitudes GP1:GP2:GP3 = 42: 18: 30 G/cm.

^{1}H-^{15}N HSQC: [4], relaxation delay 2.7 s, delay for evolution of long range couplings 60–120 ms, gradient ratios 4.8: 52.8: ± 4.8 G/cm, 16–128 scans acquired per increment, block size 4096×512. The ^{13}C and ^{1}H spectra were referenced to the ^{13}C solvent signals of d_6-DMSO, $CDCl_3$ and TMS at 39.70, 77.00, and 0.00 ppm and to the residual ^{1}H signals of d_5-DMSO, $CHCl_3$ and TMS at 2.50, 7.26 and 0.00 ppm (^{1}H), respectively. The ^{15}N spectra were referenced to the signal of liquid ammonia used as an external standard at 298 K.

Experimentally Measured Chemical Shifts

*Roemeridine (**1**)*

^{1}H-NMR (500 MHz, d_6-DMSO): 1.52 (H-16a), 1.74 (H-14a), 1.80 (H-15a, H-15b), 1.93 (H-11a), 2.08 (H-11b), 2.24 (H-29), 2.29 (H-6a), 2.33 (H-16b), 2.54 (H-25a, H-25b), 2.65 (H-5a), 2.74 (H-14b), 2.78 (H-5b), 2.91 (H-26a), 2.96 (H-27), 2.97 (H-6b), 3.12 (H-7), 3.14 (H-26b), 3.3 (H-N3, bs), 3.76 (H-31), 3.80 (H-30), 3.85 (H-28), 3.90 (H-12), 6.63 (H-3), 6.89 (H-22), 7.07 (H-19), 8.6 (OH, bs), 9.27 (H-N2)

^{13}C-NMR (125 MHz, d_6-DMSO): 23.20 (C-25), 27.20 (C-5), 32.03 (C-14)[a], 32.62 (C-15)[a], 35.62 (C-11), 38.88 (C-26), 43.58 (C-29), 47.55 (C-10), 50.63 (C-16), 54.68 (C-6), 55.72 (C-13), 56.10 (C-30), 56.28 (C-28), 56.36 (C-31), 56.95 (C-27), 64.52 (C-7), 80.31 (C-12), 96.02 (C-19), 101.02 (C-22), 108.80 (C-24), 109.00 (C-3), 119.93 (C-23), 122.01 (C-8), 129.91 (C-18), 132.56 (C-4), 134.75 (C-9), 137.87 (C-17), 139.95 (C-1), 144.23 (C-21), 146.06 (C-20), 147.82 (C-2)

[a] may be interchanged

^{15}N-NMR (50 MHz, d_6-DMSO): 37.3 (N-3), 44.7 (N-1), 121.2 (N-2)

*Armepavine (**2**)*

^{15}N-NMR (50 MHz, $CDCl_3$): 32.7 (N-2)

*Chelerythrine chloride (**3**)*

^{15}N-NMR (50 MHz, d_6-DMSO): 186.2 (N-5)

*Sanguilutine chloride (**4**)*

^{15}N-NMR (50 MHz, d_6-DMSO): 186.6 (N-5)

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