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Differentiation of Regioisomers at ^{15}N Natural Abundance Using Gradient-Enhanced $^{1}H/^{15}N$ HMBC NMR Spectroscopy

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ABSTRACT: The $^{1}H/^{15}N$ HMBC experiment at ^{15}N natural abundance using pulsed field gradients is a useful tool for chemical shift assignment and structure elucidation. This experiment assisted in the identification of regioisomers that could not be distinguished with conventional $^{1}H/^{1}H$ nuclear Overhauser or $^{1}H/^{13}C$ correlation NMR experiments. The natural abundance ^{15}N gradient-enhanced HMBC experiment was easily implemented, provided high-quality spectra and readily distinguished nitrogen-containing regioisomers. This experiment can aid in structure elucidation when traditional ^{1}H and ^{13}C experiments fail to provide unique structural assignment. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{15}N NMR; natural abundance; gradient HMBC

INTRODUCTION

Long-range heteronuclear chemical shift correlation experiments (HMBC)[1,2] are extremely useful for structure elucidation and assignment information. These experiments yield proton connectivities through quaternary carbons or heteronuclei and recently have improved in sensitivity with the incorporation of pulsed field gradients.[3,4] The addition of pulsed field gradients into NMR pulse sequences yields spectra with fewer artifacts and decreases the data collection because the selection of the desired coherence pathways occurs without extensive phase cycling. Although the HMBC experiment is widely applied to organic structure determination at ^{13}C natural abundance,[5–13] only a few applications exist for this experiment at ^{15}N natural abundance using pulsed field gradients.[14,15] We report here the application of the $^{1}H/^{15}N$ HMBC experiment at natural abundance ^{15}N using pulsed field gradients as a tool to differentiate regioisomers that cannot be distinguished with conventional $^{1}H/^{1}H$ nuclear Overhauser or $^{1}H/^{13}C$ correlation NMR experiments. The natural abundance ^{15}N gradient-enhanced HMBC experiment is easy to implement, provides high-quality spectra and readily distinguishes nitrogen-containing regioisomers. Because the availability of pulsed field gradient technology has increased, this experiment should be implemented routinely for structure elucidation.

The regioisomers **1** and **2** in Fig. 1 are easily identified utilizing two-dimensional (2D) ^{1}H correlated spectroscopy (COSY) and 2D nuclear Overhauser spectroscopy (NOESY) experiments. These structures are related to tepoxalin,[16–18] which is an anti-inflammatory agent. Proton pairs 11 and 7 for regioisomer **1** and 11 and 16 for regioisomer **2** exhibited a nuclear Overhauser effect (NOE) and assigned the two conformers.

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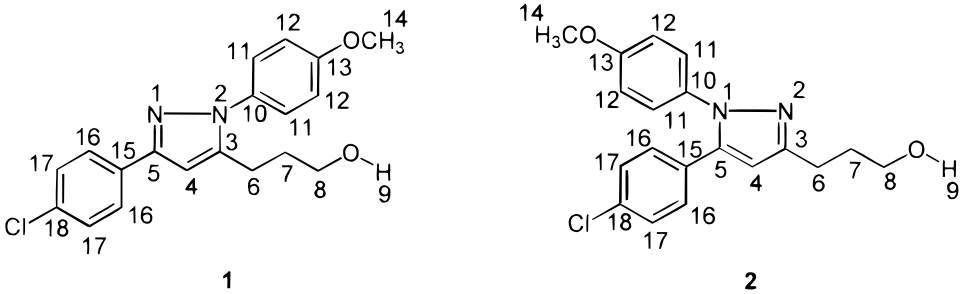

Figure 1. Regioisomers **1** and **2** can be distinguished with 2D COSY and NOESY experiments. The numbering in the figure corresponds to the NMR assignments and not to the IUPAC numbering for the compounds. The ortho and meta protons in the chlorophenyl ring have the same numbering because the spins are equivalent on the NMR time-scale.

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The reaction of 6-(4-chlorophenyl)hexanoic acid-4,6-dione with 2-hydrazinopyridine dihydrochloride produces regioisomers **3** and **4** (Fig. 2). Because the 2-position in the aromatic ring of compounds **3** and **4** has a nitrogen instead of a CH, conventional homonuclear and ^{13}C heteronuclear experiments failed to confirm the identity of the regioisomers. Proton pairs 11 and 7 for regioiosmer **3** and 11 and 17 for regioisomer **4** did not exhibit an NOE to assign the two conformers. The ^{15}N natural abundance gradient-enhanced HMBC experiment provided unambiguous spectral assignment of the two regioisomers.

RESULTS AND DISCUSSION

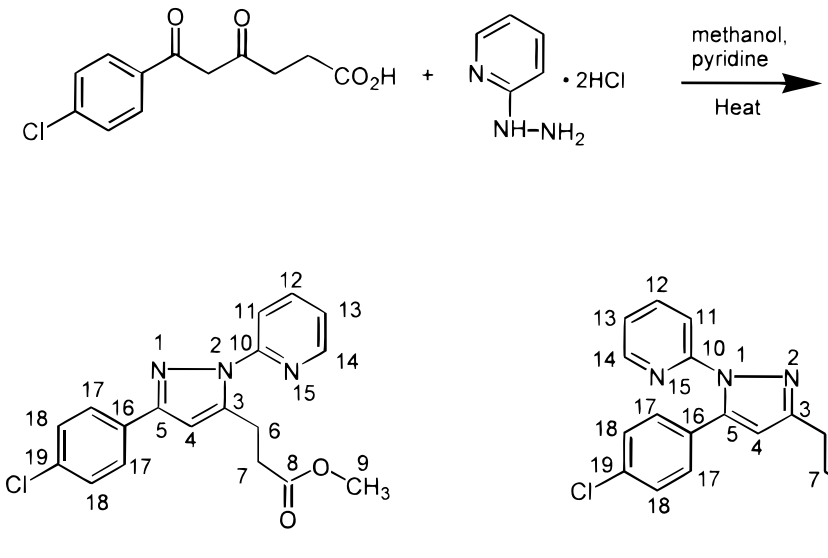
^{1}H assignments for **3** and **4** were made with ^{1}H one-dimensional (1D) spectra and 2D COSY spectra. The chemical shifts and coupling constants for **3** and **4** are given in Table 1. No significant differences in chemical shift or coupling constant exist for the two regioisomers and their unequivocal assignment could not be made with conventional 2D ^{1}H NOESY. The 2D $^{1}H/^{13}C$ HMBC experiment did not yield the connectivities which could distinguish **3** from **4**; however, we were able to use gradient-enhanced $^{1}H/^{15}N$ HMBC experiments to identify unique connectivites for each regioisomer. The experiment was easily implemented at ^{15}N natural abundance because the compounds were readily soluble in chloroform and possessed large, long-range $^{1}H/^{15}N$ couplings of the pyrazole ring.

The 2D gradient-enhanced $^{1}H/^{15}N$ HMBC spectra at natural abundance for **3** and **4** are shown in Fig. 3. The signal-to-noise ratio in both spectra was good and resonance assignments for the nitrogens were made based on long-range couplings. The two nitrogens in the pyrazole ring were identified via the three-bond coupling from H-4. For **3**, H-4 had three-bond connectivities to the nitrogens N-1 and N-2 at 296.38 and 217.73 ppm. For **4**, H-4 had three-bond connectivities to the nitrogens N-1 and N-2 at 214.19 and 304.16 ppm. The important correlation which distinguished N-1 and N-2 in both regioisomers and determined the placement of the pyridine ring was the four-bond correlation from H-12. H-12 has a four-bond connectivity to N-2 at 217.73 ppm in **3** and to N-1 at 214.19 ppm in **4**. Also

Table 1. Proton and nitrogen chemical shifts of compounds 3 and 4[a]

	3		4	
Position	^{1}H	^{15}N	^{1}H	^{15}N
1	—	296.38	—	214.19
2	—	217.73	—	304.16
4	6.27 (s)	—	6.08 (s)	—
6	3.52 (t)	—	3.04 (t)	—
7	2.65 (t)	—	2.69 (t)	—
9	3.34 (s)	—	3.37 (s)	—
11	7.95 (d)	—	7.67 (d)	—
12	7.16 (t)	—	7.09 (t)	—
13	6.52 (t)	—	6.45 (t)	—
14	8.06 (d)	—	7.85 (d)	—
15	—	285.46	—	294.85
17	7.71 (d)	—	7.03 (d)	—
18	7.23 (d)	—	6.96 (d)	—

[a] Coupling constants (Hz): **3**, $J_{6,7} = 7.6$, $J_{11,12} = 8.3$, $J_{13,14} = 4.3$, $J_{17,18} = 8.1$; **4**, $J_{6,7} = 7.5$, $J_{11,12} = 8.1$, $J_{13,14} = 4.2$, $J_{17,18} = 8.1$.



3 **4**

Figure 2. Reaction of 6-(4-chlorophenyl)hexanoic acid-4,6-dione with 2-hydrazinopyridine dihydrochloride to produce regioisomers **3** and **4**. The numbering in the figure corresponds to the NMR assignments and not to the IUPAC numbering for the compounds. The ortho and meta protons in the chlorophenyl ring have the same numbering because the spins are equivalent on the NMR time-scale.

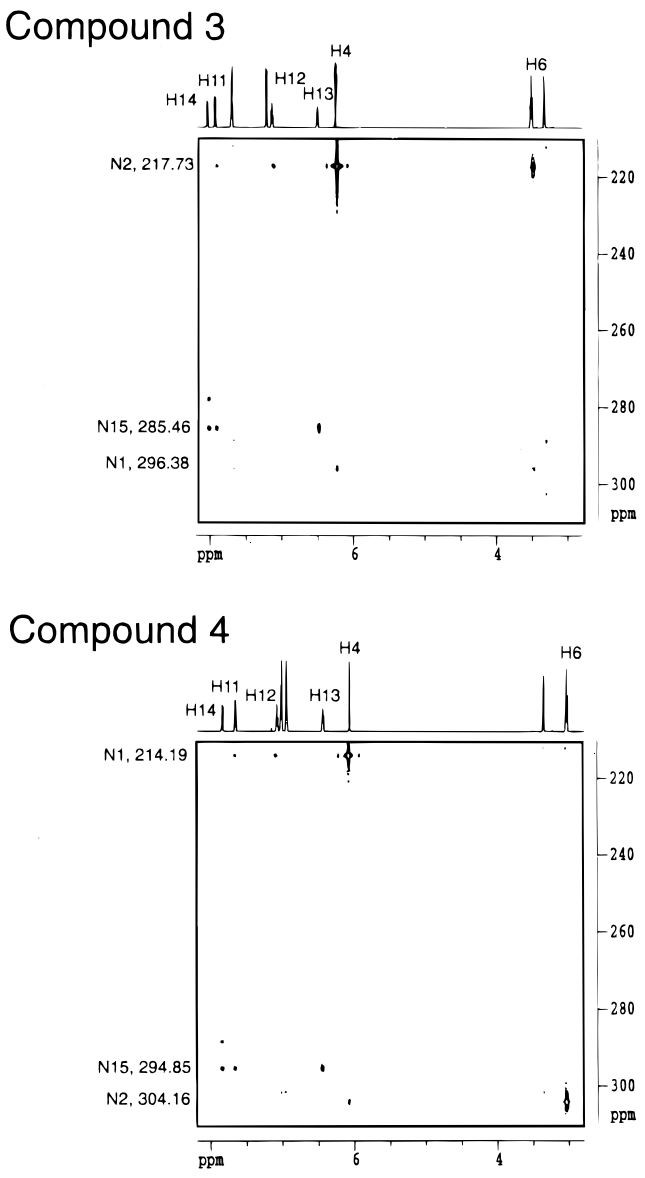


Figure 3. Sections of the gradient-enhanced natural abundance $^{1}H/^{15}N$ HMBC spectra of **3** and **4**.

observed is a three-bond connectivity from H-11 to N-2 at 217.73 ppm and N-1 at 214.19 ppm in **3** and **4**, respectively. These three- and four-bond connectivities from the pyridine ring establish the assignments of N-1 and N-2. The four-bond coupling from H-17 to N-1 is not observed in either compound, implying that long-range $^{1}H/^{15}N$ couplings in heterocyclic rings do not always follow predictable rules. It is also highly unlikely that a five-bond connectivity from H-13 to the pyrazole would be observed.

Further support for the assignments of N-1 and N-2 in **3** and **4** resulted from the three-bond coupling from H-6. H-6 had a three-bond coupling to N-2 at 217.73 ppm for **3** and a three-bond coupling to N-2 at 304.16 ppm for **4**. Apparently in **4**, N-2 at 304.16 ppm is deshielded by the pyridine ring because of its downfield chemical shift. A similar observation of deshielding of the pyridine ring occurs for N-1 at 296.38 ppm in **3**. The deshielding of a pyrrole-type nitrogen connected to a pyridine-type nitrogen agrees with empirical observations[19,20] and with quantum mechanical calculations.[21] The resonances at 285.46 and 294.85 ppm were assigned to the pyridine nitrogens (N-15) of **3** and **4**, respectively. The HMBC two- and three-bond connectivities between the aromatic protons H-11, H-13 and H-14 and N-15 confirmed these assignments. The correlation peak at approximately 8.1, 277.8 ppm in the spectrum for **3** is probably a minor impurity in the sample because a similar correlation peak appears at 7.9, 288.0 ppm in the spectrum for **4**. Other peaks in both spectra are due to noise or t_1 streaking.

CONCLUSION

The HMBC experiment is routinely applied to organic structure determination at ^{13}C natural abundance and can easily be implemented at ^{15}N natural abundance. This experiment should be utilized more for ^{15}N chemical shift assignment and structure elucidation. The gradient-enhanced natural abundance $^{1}H/^{15}N$ HMBC experiment provided long-range connectivities through quaternary nitrogens and aided in the assignment of regioisomers.

EXPERIMENTAL

Syntheses

Compounds 1 and 2. A solution of 6-(4-chlorophenyl)-1-hydroxyhexane-4,6-dione (48.2 g, 200 mmol) and 4-methoxyphenylhydrazine hydrochloride (35.0 g, 200 mmol) in a mixture of methanol (100 ml) and pyridine (20 ml) was stirred under nitrogen at ambient temperature for 1.5 h. The mixture was concentrated *in vacuo* and partitioned between chloroform (300 ml) and 1 M HCl (300 ml). The chloroform layer was dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The crude oil was decolorized (Norit) in hot diethyl ether (300 ml) and filtered through a pad of Celite. The cooled filtrate afforded, in two crops, 58.7 g (86%) of **2**, 5-(4-chlorophenyl)-3-hydroxypropyl-1-(4-methoxyphenyl)pyrazole, as a slightly off-white powder, m.p. 87.5–88 °C; calculated for $C_{19}H_{19}ClN_2O_2$, C 66.56, H 5.59, N 8.17; found, C 66.54, H 5.76, N 8.02%; IR, 3320, 1495 cm^{-1}; chemical ionization mass spectrometry (CIMS) *m/z* 343, MH^+. Compound **1** was obtained by crystallization of the filtrate derived from **2** in diethyl ether. Recrystallization afforded 3.69 g (5.4%) of **1**, 3-(4-chlorophenyl)-5-(3-hydroxypropyl)-1-(4-methoxyphenyl)pyrazole, as a slightly off-white powder, m.p. 108.5–110 °C; calculated for $C_{19}H_{19}ClN_2O_2$, C 66.56, H 5.59, N 8.17; found, C 66.58, H 5.64, N 8.24%; IR, 1410, 1045 cm^{-1}; CIMS, *m/z* 343, MH^+.

Compounds 3 and 4. A solution of 6-(4-chlorophenyl)hexanoic acid-4,6-dione (1.27 g, 5.0 mmol) and 2-hydrazinopyridine dihydrochloride (0.91 g, 5.0 mmol) in a mixture of methanol (25 ml) and pyridine (2.0 ml) was stirred under nitrogen at ambient temperature for 12 h and then heated at reflux for 2 days. The mixture was concentrated *in vacuo* and partitioned between ethyl acetate (150 ml) and water (250 ml). The ethyl acetate layer was washed once with brine (200 ml) and dried over anhydrous sodium sulfate. Filtration and concentration of the solution afforded the crude product (1.75 g), a mixture of two components by TLC [solvent: hexane–ethyl acetate (3:1)] as a light-yellow oil. The crude mixture was purified by flash chromatography with the same solvent system on silica gel and afforded, in order of

elution, two very light-yellow solids, **3** and **4**. Compound **3**: methyl 3-[3-(4-chlorophenyl)-1-(2-pyridyl)pyrazol-5-yl]propionate, 0.25 g (15%), m.p. 60–60.5 °C); calculated for $C_{18}H_{16}ClN_3O_2$, C 63.25, H 4.72, N 12.30; found, C 63.12, H 4.70, N 12.14%; IR, 1744 cm^{-1}; CIMS, *m/z* 342, MH^+. Compound **4**: methyl 3-[5-(4-chlorophenyl)-1-(2-pyridyl)pyrazol-3-yl]propionate, 0.72 g (44%), m.p. 71–72 °C; calculated for $C_{18}H_{16}ClN_3O_2$, C 63.25, H 4.72, N 12.30; found, C 63.34, H 4.64, N 12.28%; IR, 1737 cm^{-1}; CIMS, *m/z* 342, MH^+.

NMR Spectroscopy

All ^1H NMR spectra were recorded on a Bruker DMX 600 MHz spectrometer at 298 K using a triple-resonance probe ($^1H/^{13}C/^{15}N$) equipped with triple axis gradients (Accustar). All samples consisted of approximately 50 mg of **3** or **4** in 0.6 ml of chloroform-*d*. Chemical shifts were measured relative to the TMS signal at 0.00 ppm. One-dimensional ^1H NMR spectra were collected with 8K complex points and a sweep width of 7042 Hz.

Two-dimensional (2D) gradient enhanced $^1H/^{15}N$ HMBC experiments at natural abundance were obtained with spectral widths of 4807 Hz in the ^1H dimension and 7299 Hz in the ^{15}N dimension. Three *z*-axis gradient pulses 1 ms in length were applied using a gradient ratio of 70:30:50. The gradient strength measured on the *z*-axis gradient at 10% gradient output was calculated to be *ca.* 6.8 G cm^{-1} A^{-1}. A 150 ms delay occurred after each gradient pulse. The first two gradient pulses (70 and 30%) were placed on either side of the ^1H 180° pulse in the center of the t_1 evolution period.[1] The final gradient pulse (50%) occurred after the last ^{15}N 90° pulse. The 2D spectra were acquired in the magnitude mode with 2048 complex points collected in t_2 and 256 t_1 increments of 128 transients each. The ^{15}N carrier position was at 250 ppm relative to liquid NH_3, 25 °C at 0.0 ppm. Two-dimensional data sets were processed using Bruker software. Matrix files were 2048 × 512 points in size. The t_2 and t_1 time domain transforms were weighted with a sine-shaped function shifted by 90° and 45°, respectively. A t_2 noise subtraction was performed.

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