Supporting information: Köck, Junker, Lindel "Impact of the $^{1}H,^{15}N$-HMBC Experiment on the Constitutional Analysis of Alkaloids"

1) ^{15}N-NMR spectroscopy

The ^{14}N isotope, which exists in 99.63% natural abundance, is rarely used in NMR spectroscopy because of the relatively broad lines due to its quadrupole moment. The ^{15}N isotope with a spin of ½ has no restrictions due to the line widths of the signals, but the relative sensitivity of ^{15}N against ^{1}H is only $3.05 \cdot 10^{-6}$ and only 0.022 in comparison with ^{13}C. The application of a 1D ^{15}N-NMR spectrum is therefore very difficult because of the usually small quantities of natural products.

The following characteristics of the ^{15}N isotope are disadvantageous in comparison to ^{13}C for NMR investigations:

a) the natural abundance of the ^{15}N isotope is 0.37% approximately 1/3 that of ^{13}C,

b) the gyromagnetic ratio of ^{15}N is about 2/5 of ^{13}C and

c) the relaxation times of ^{15}N are longer in comparison to ^{13}C.

The referencing of the ^{15}N chemical shifts is more difficult than for ^{13}C because standardly used solvents do not contain ^{15}N with the exception of DMF. An external standard such as nitromethane (0 ppm) can be used. Because of the insensitivity of the ^{15}N nuclei the pulse width calibration on the ^{15}N channel requires an extra samples which should be ^{15}N enriched. The signal-to-noise (S/N) ratio of a natural abundance sample at standard concentrations is too low for pulse width calibration.

2) History of the $^{1}H,^{15}N$-HMBC

The general utility of the HMBC experiment is clearly reflected by the application to several complex molecules shortly after its appearance in the literature.[1] The $^{1}H,^{15}N$-HMBC was first applied in 1988 to a DNA-binding protein[2] and in 1990 to ^{15}N labeled human thioredoxin[3]. The first application to an alkaloid was also described in 1990.[4] In 1995, a comprehensive review article on the $^{1}H,^{15}N$-HMBC experiment was published.[5] Despite the potential in structure elucidation of alkaloids and its established experimental setup, the ^{15}N-based experiment is not as widely used as the $^{1}H,^{13}C$-HMBC. This is very astonishing because several of the first applications of the proton-detected multiple quantum coherence experiments (HMQC) were applied to ^{15}N.[6] In contrast to oxygen-rich compounds, alkaloids have the advantage that the ^{15}N isotope is accessible to 2D correlation experiments. NMR experiments sensitive to ^{17}O can usually not be applied to natural products. The recent developments of the HMBC experiment[7] are not discussed here.

3) Practical Aspects of the $^{1}H,^{15}N$-HMBC

For the $^{1}H,^{15}N$-HMBC experiment (proton excitation and detection) only the natural abundance of ^{15}N is of relevance for the sensitivity. Therefore, the $^{1}H,^{15}N$-HMBC experiment is 3 times less sensitive than the $^{1}H,^{13}C$-HMBC experiment leading to a theoretical increase of the measuring time by a factor of 9 (in practice the measuring time of a $^{1}H,^{15}N$-HMBC is about 6 times longer). The increased measuring time is not a problem for a natural product sample of about 20 mg because a $^{1}H,^{13}C$-HMBC takes about 30 to 60 minutes. Usually the relaxation delay is set approximately 500 ms longer as for the $^{1}H,^{13}C$-HMBC (relaxation delay and acquisition time 2.5 to 3.0 s). The introduction of pulsed field gradients[8] was especially valueable for the broad application of the HMBC experiment[9] (even more important for the ^{15}N version) since before the use of pulsed field gradients only phase cycling was available for the suppression of protons bound to ^{12}C or ^{14}N. Because of this, HMBC spectra usually had very strong t_1 noise which made the analysis without a t_1 noise reduction (e. g., skyline projection) almost impossible.

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