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^{15}N Inverse-Detected NMR Spectra of 1-Phenylazo-2-Naphthol

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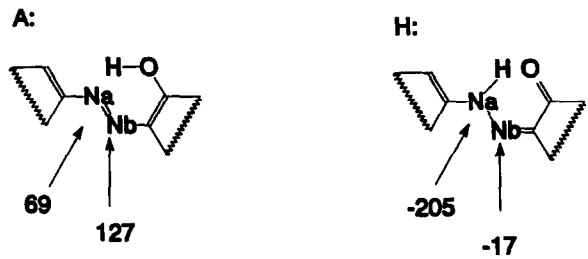
ABSTRACT

*One-dimensional ^{15}N proton-detected (inverse) NMR spectra of 1-phenylazo-2-naphthol (**1**) were measured in deuterochloroform at concentrations in the range 1 mol litre^{-1} to 10^{-5} mol litre^{-1} in the ^{15}N selective enriched compound **1a** (95% $^{15}N_a$), and at concentrations in the range 1 mol litre^{-1} to 10^{-3} mol litre^{-1} in compound **1b** having the natural abundance level of ^{15}N isotope. Two-dimensional spectra of the same compounds were also determined. The results obtained can serve as model data to evaluate the possibilities of inverse detection in the ^{15}N NMR spectra of azo dyes.*

1 INTRODUCTION

Azo-hydrazone tautomerism is a characteristic feature of azo dyes.[1–3] NMR spectroscopy is a particularly advantageous method for the quantitative evaluation of this tautomerism.[3] Many NMR experiments can be used for this purpose,[4] but ^{15}N NMR spectra are preferred because of the existence of large differences in the ^{15}N chemical shifts of the azo (A) and hydrazone (H) forms in azo dyes containing an intramolecular hydrogen bond:

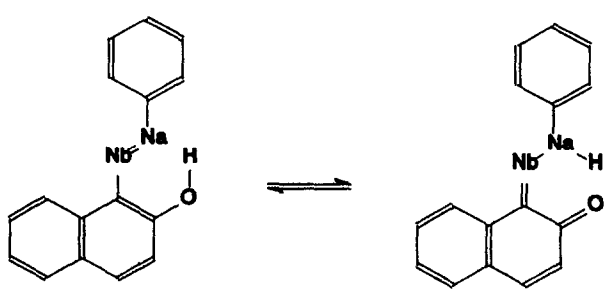
$\delta(^{15}N)$:



Differences in $\delta(^{15}N)$ for nitrogens N_a and N_b are thus *c.* 274 and 144 ppm, respectively,[5] the half-width lines of signals being in lengths of ppm, i.e. 0·1–0·4 ppm. Moreover, two sets of data are obtained (for N_a and N_b), which are practically identical and 'intrinsic' control is thus possible (cf. other methods, such as UV–VIS, in which only one value pertinent to azo-hydrazone tautomerism is obtained).

The main disadvantage of ^{15}N NMR spectroscopy consists in the low sensitivity of such measurements due to the low natural abundance level of the ^{15}N isotope (0·365%) and usually long relaxation times.[7–10] For this reason, most ^{15}N NMR studies for dyes have been carried out using rather concentrated solutions (about 10% or more). Applications of dyes, and especially the measurements of electronic spectra in the visible region are effected at concentrations several orders of magnitude lower, e.g. 10^{-4} to 10^{-5} mol litre^{-1}. It would be very advantageous to measure ^{15}N NMR spectra at concentrations that were at least comparable with these values, in order to compare the results obtained. In principle, indirect (inverse) detection[11] should provide the required increase in the sensitivity of ^{15}N NMR measurements.

The aim of this work was to test experimentally the application of the indirect detection technique to a classical tautomeric system, typified by the coupling product of aniline with 2-naphthol, i.e. **1**.



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The ^{15}N enriched compound **1a** (95% $^{15}N_a$) and the product with a natural abundance level of ^{15}N isotope **1b** (0·365%) were used for the measurements.

2 EXPERIMENTAL

1-Phenylazo-2-naphthol **1b** was prepared as reported in the literature.[12] The $^{15}N_a$ (95% ^{15}N) isotopomer **1a** was prepared analogously using ^{15}N-aniline (95% ^{15}N; Isocommerz Berlin).

Inverse ^{15}N NMR spectra were obtained using a 5 mm inverse detection probe on a Varian Unity-500 NMR spectrometer at 499·843 MHz (^1H). The decoupler channel was tuned for ^{15}N (50·653 MHz) and the standard heteronuclear multiple quantum coherence (HMQC) pulse sequence of Summers *et al.*[13] in a one-dimensional version was used. The decoupler ^{15}N channel was equipped with 50·65 MHz band pass and 76·75 MHz reject filters; the ^2H lock channel was modified to include a 76·75 MHz narrow band pass filter to avoid disturbances of the lock by the ^{15}N irradiation. The ^1H observe channel was equipped with a 375 MHz high pass filter.

The HMQC pulse sequence in the one-dimensional version is a heteronuclear spin–echo difference experiment. Its essence is the cancellation of the signals from protons attached to ^{14}N, leaving only the signals from protons attached to ^{15}N. BIRD pulse (BIlinear Rotation Decoupling) was used to improve the cancellation of ^1H–^{14}N signals.[13] The full sequence is illustrated as follows:

$$^1H: 90°(x)-\Delta-180°(x)-\Delta-90°-(x)-\text{null}-90°(x)-\Delta-180°(x)-\Delta-\text{acq}\ (x, -x)$$

$$^{15}H: \qquad 180°(x) \qquad\qquad 90°(x, -x)\ \ 90°(x)\ \ \text{dec.}$$

The BIRD null delay was optimized at 0·6 s, relaxation delay was 3 s, spectral width 6000 Hz, proton 90° pulse width 18 μs, nitrogen 90° pulse width 53 μs, and delay $\Delta = 1/2J_{NH} = 1/132$ s.

A sample of 1-phenylazo-2-naphthol **1a** (95% ^{15}N enriched) was dissolved in $CDCl_3$ and the 1D HMQC ^1H-{^{15}N} experiment was carried out at different concentrations (from 1 mol litre^{-1} to 10^{-5} mol litre^{-1}). The number of transients was set with respect to the dye concentration; 256 transients were used for the most dilute solution (10^{-5} mol litre^{-1}).

1D HMQC ^1H-{^{15}N} spectra of compound **1b** at the natural abundance of ^{15}N were measured with sample concentrations of 1, 0·1 and 0·01 mol litre^{-1} and the number of transients 16, 64 and 256, respectively.

Total experimental time was less than 20 min even for the lowest sample concentration. To measure 0·001 mol litre^{-1} solution, 1536 pulses (2 h of accumulation, S/N *c.* 6) or 12 800 pulses (10·5 h of accumulation, S/N *c.* 16) were applied.

Parameters for the 2D HMQC ^{1}H-{^{15}N} spectra of compounds **1a**, **b** are given in the legends to Figs 3 and 4. The same pulse sequence (in its 2D version) mentioned above was used for measurements either without (Fig. 3) or with ^{15}N decoupling (Fig. 4).

The ^{1}H and ^{15}N chemical shifts are referenced to internal tetramethylsilane and external nitromethane (δ = 0·0).

3 RESULT AND DISCUSSION

In Table 1, theoretical sensitivities of various ^{15}N NMR experiments are compared as a function of different types of excitations and detections. The sensitivity of inverse experiments is the highest possible, which is why we have decided to use this technique for the measurement of ^{15}N NMR characteristics of very diluted solutions of azo dyes.

Figure 1(a) shows 1D ^{1}H NMR spectrum and Fig. 1(b)–(f) 1D ^{15}N proton-detected NMR spectra of ^{15}N-enriched (95% ^{15}N) compound **1a** together with the concentrations of compound measured, number of pulses applied and signal-to-noise ratios.

TABLE 1
Comparison of Theoretical Sensitivities of Various NMR Experiments as a Function of Different Excitations and Detections[a]

Experiment	*S/N*[b]	*Amplification factor*	^{15}N[c]
(a) Direct observation	$\gamma_N(\gamma_N)^{3/2}$		1·0
(b) INEPT	$\gamma_H(\gamma_H)^{3/2}$		
Compared with (a)		$\gamma_H(\gamma_N)$	9·9
(c) Inverse INEPT	$\gamma_N(\gamma_H)^{3/2}$		
Compared with (a)		$\gamma_H(\gamma_N)^{3/2}$	31·0
Compared with (b)		$\gamma_H(\gamma_N)^{1/2}$	3·1
(d) Inverse shift correlation	$\gamma_H(\gamma_H)^{3/2}$		
Compared with (a)		$\gamma_H(\gamma_N)^{5/2}$	306·0
Compared with (b)		$\gamma_H(\gamma_N)^{3/2}$	31·0

[a] Taken from Ref. 14.
[b] Signal-to-noise ratio.
[c] Relative sensitivity.

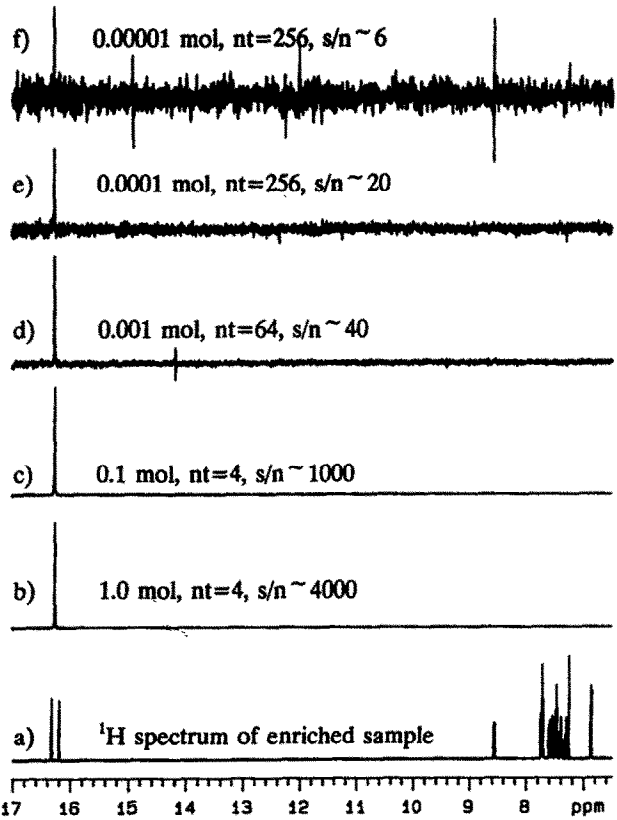

Fig. 1. One-dimensional HMQC ^{1}H-{^{15}N} spectra of $^{15}N_a$-enriched (95% ^{15}N) compound **1a** in $CDCl_3$ at 300 K (concentrations are given in mol litre^{-1}; *nt* = number of transients, *s/n* = signal-to-noise ratio).

The signal for acidic proton ($\delta(^1H) = 16{\cdot}11$) is split into a doublet with $^1J(^{15}N, ^1H) = 63{\cdot}7$ Hz (Fig. 1(a)). The ^{15}N resonance frequency of N_a nitrogen was determined from the standard 1D ^{15}N NMR spectrum. Using these values, 1D HMQC ^{1}H-{^{15}N} spectra were measured at different concentrations (Fig. 1(b)–(f)). The creation of heteronuclear multiple-quantum coherence followed by reconversion to detectable single-quantum proton coherence is based on $^1J(^{15}N, ^1N)$ and, thus, only the signal corresponding to a $^{15}N_a/^1H$ pair is obtained while other signals are suppressed. A weak signal can be obtained even at a concentration of 0·000 01 mol litre^{-1} for compound **1a**, i.e. for 2·483 μg of compound **1a** per ml of solution, in a very short time.

Similar results as for **1a** are shown in Fig. 2(a)–(e) for compound **1b** measured at the natural abundance level of ^{15}N (0·365%). The signal for acidic proton, prevailingly bonded to ^{14}N ($I = 1$) due to azo–hydrazone tautomerism, is a broadened singlet with *c.* 0·18% ^{15}N satellites

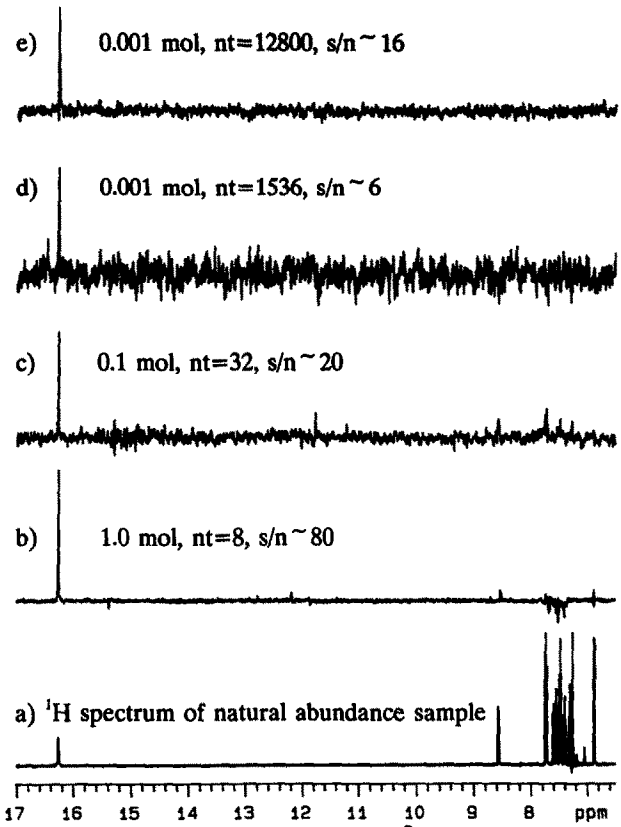

Fig. 2. One-dimensional HMQC ^{1}H-{^{15}N} spectra of compound **1b** at the natural abundance of ^{15}N in $CDCl_3$ at 300 K (concentrations are given in mol litre^{-1}; *nt* = number of transients, *s/n* = signal-to-noise ratio).

(Fig. 2(a)). The suppression of 99·635% of the proton signal associated with the ^{14}N/^{1}H arrangement was necessary to obtain signals in Fig. 2(b)–(e).

The data reported in Figs 1 and 2 can be used to estimate the number of scans required for a two-dimensional HMQC ^{1}H/^{15}N NMR experiment at a given concentration. In a typical case, the ^{15}N resonance frequency is, of course, not known beforehand and a ^{15}N spectral width covering the possible resonance frequency region must be used.

Figure 3 shows the two-dimensional HMQC ^{1}H/^{15}N spectrum of ^{15}N-enriched (95% ^{15}N) compound **1a** in $CDCl_3$ at 300 K measured without ^{15}N decoupling at a concentration of 0·0001 mol litre^{-1}. Both $\delta(^{15}N_a)$ and $^1J(^{15}N, ^1H)$ can be read from the appropriate projections.

A part of the two-dimensional HMQC ^{1}H/^{15}H spectrum of compound **1b** (0·1 mol litre^{-1}) in $CDCl_3$ at 300 K measured with ^{15}N decoupling is shown in Fig. 4.

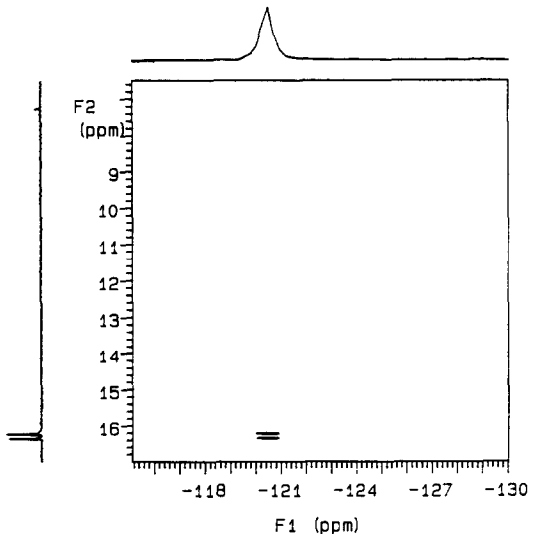

Fig. 3. Part of the two-dimensional HMQC ^{1}H-{^{15}N} spectrum of $^{15}N_a$-enriched (95% ^{15}N) compound **1a** (0·0001 mol litre^{-1}) in $CDCl_3$ at 300 K (499·843/50·653 MHz; 6000 Hz (F_2) ×3000 Hz (F_1); relaxation delay 3 s; acquisition time 0·299 s; number of increments 128; number of transients 32; experimental time 10·5 h).

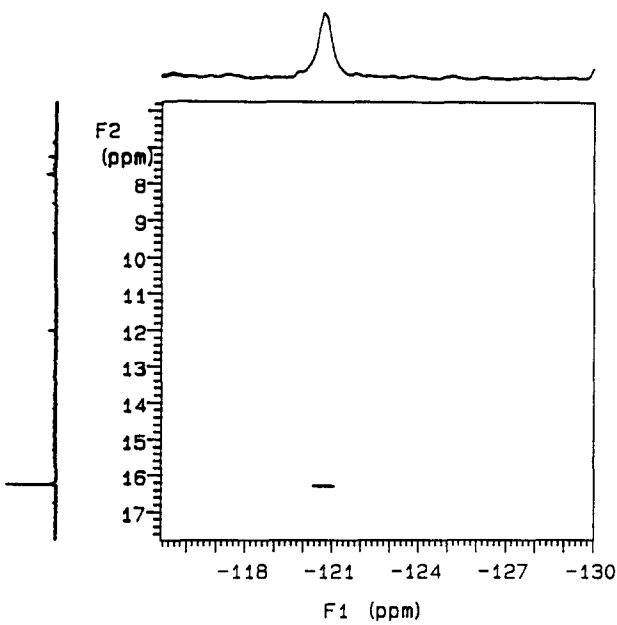

Fig. 4. Part of the two-dimensional HMQC ^{1}H-{^{15}N} spectrum of compound **1b** (0·01 mol litre^{-1}) in $CDCl_3$ at 300 K (number of transients 16; experimental time 2·5 h; other conditions were the same as those reported in Fig. 3).

4 CONCLUSIONS

The data in Figs 1–4 indicate that the indirect one- and two-dimensional HMQC $^{1}H/^{15}N$ NMR spectra of azo dyes can be successfully used to measure ^{15}N chemical shifts and $^{1}J(^{15}N_a, ^{1}H)$ even in very dilute solutions.

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