Synthesis and Multinuclear NMR Relaxation Study of 2-Phenyldiazene-1-carbonitrile 2-Oxide and its [^{15}N]-labelled Isotopomers

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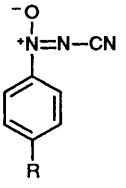
[^{15}N]-2-Phenyldiazene-1-carbonitrile 2-oxide has been synthesized using nitrosobenzene and [^{15}N]-NH_2CN and fully characterized by mass spectrometry and IR, ^{13}C and ^{15}N NMR spectroscopy. ^{13}C and ^{15}N spin–lattice relaxation times were measured and are discussed in terms of two main relaxation mechanisms: dipole–dipole (DD) and chemical-shift anisotropy (CSA). Quantitative evaluation of the two contributions was achieved by measuring the nuclear Overhauser effect (NOE) and by performing experiments at different magnetic-field strengths (4.7, 6.3 and 9.4 T). The availability of ^{15}N selectively-labelled isotopomers provided a simple method to estimate, from ^{13}C–T_1 data, C–N bond distances. Motional parameters for isotropic molecular motion and phenyl internal rotation were calculated from relaxation data for the protonated ring carbons.

In earlier papers[1–3] the cytotoxic activity of a series of analogues of 'Calvatic acid' 1,[4] was described.



1 R = CO_2H
2 R = H

Structure–activity relationships in a series of aryl and heteroaryl derivatives R–N(O)=N–X, where X is an electron-withdrawing group, showed that the –N(O)=N–CN function is a powerful inductor of activity, useful in the design of potential antitumoral and antimicrobial compounds.[5,6]*

More recent studies on electronic and hydrophobic properties of this function have also been carried out. In particular, the electronic σ constant,[7] the hydrophobic π constant[7] and the dipole moment[8] were evaluated, indicating that in many respects the physicochemical properties of the ONN–CN group closely parallel those of the nitro group.

In order to complete the structural and physicochemical characterization of the –N(O)=NCN moiety, we undertook a detailed ^{13}C and ^{15}N NMR spectroscopic study on 2-phenyldiazene-1-carbonitrile 2-oxide (**2**). It is well known that, in particular, spin–lattice relaxation time (T_1) measurements represent one of the most powerful tools for obtaining structural and dynamic information on molecules in solution. The availability of selectively-labelled ^{15}N isotopomers of the title compound (**2a** and **2b**) allowed us to exploit the large amount of information available from the relaxation data by measuring ^{13}C and ^{15}N T_1s on both labelled and unlabelled molecules at different magnetic-field strengths.

Experimental

[^{15}N]-2-Phenyldiazene-1-carbonitrile 2-oxide was synthesized from nitrosobenzene and [^{15}N]cyanamide following the procedure described in ref. 9. ^{15}N-Enriched cyanamide was supplied by *Spectrometrie Spin et Techniques*. Nitrosobenzene and *N,N*-dimethylaniline were commercial samples (Aldrich) and were used without further purification.

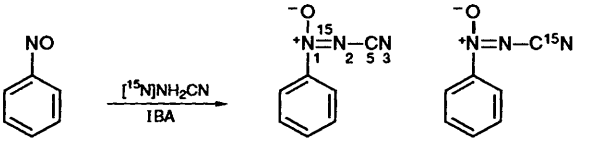
Mass and IR spectra were performed on a Varian CH7 MAT and IFS 113V Bruker instruments, respectively. Solutions for NMR measurements were prepared in [$^2H_6$]acetone and were not degassed. ^{13}C and ^{15}N NMR spectra were recorded on a JEOL EX-400 spectrometer at 100.5 and 40.5 MHz, respectively. T_1 values were recorded at 4.7, 6.3 and 9.4 T with a Bruker AC-200, a JEOL GX/270 and a JEOL EX-400, respectively, at 300 K. The inversion recovery pulse method was utilized and the data analysed by a three-parameter fit procedure. Typically 12–15 τ values were used for each determination, with a delay $\geqslant 7T_1$ between $180 \geqslant \tau/s \geqslant 90$ pulse sequences and digital resolution better than 1 Hz per data point. The measurements were repeated at least three times and the accuracy estimated as better than 3% for ^{13}C and 5–10% for ^{15}N T_1 values. All spectra were obtained under proton-decoupling conditions.

NOE factors were determined using a standard procedure.[10] The delay between pulses was in all cases at least $10T_1$ and the results represent the average of three measurements, with a precision of $\pm 5\%$.

Results and Discussion

Synthesis and Structure Characterization of ^{15}N-*Enriched* 2-*Phenyldiazene-1-carbonitrile 2-Oxide.*—The synthesis of the enriched [^{15}N]-2-phenyldiazene-1-carbonitrile 2-oxide, using nitrosobenzene and [^{15}N]cyanamide in the presence of (diacetoxyiodo)benzene, has been carried out according to the procedure we recently proposed[9] (Scheme 1).

The ^{15}N-enriched cyanamide used was 90% selectively



2a **2b**

Scheme 1

* Ref. 5 was published containing some errors. The compounds reported in Table 1 have progressive numbers: 8–13.

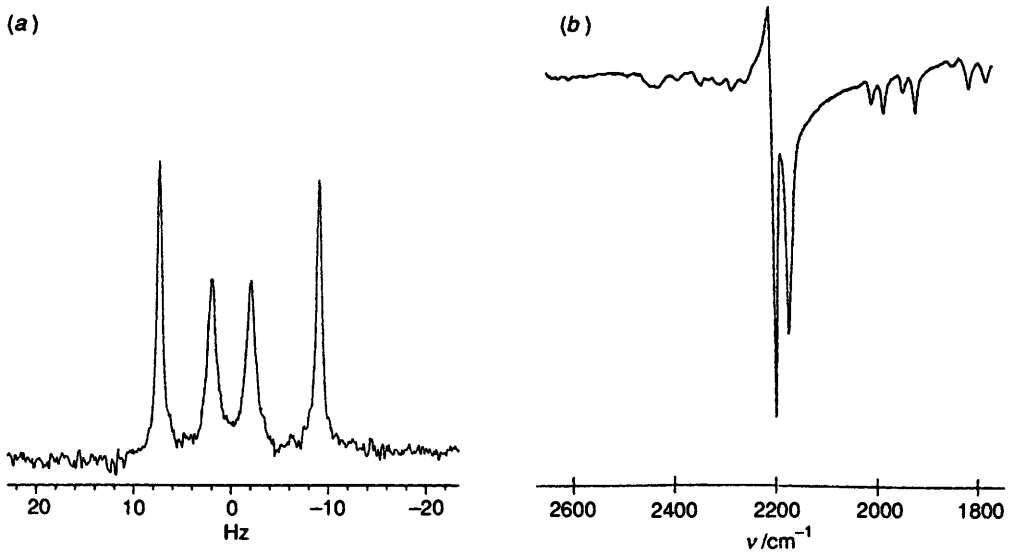


Fig. 1 (*a*) ^{13}C NMR spectrum of C(5) in an equimolar mixture of **2a** and **2b** recorded at 100.5 MHz and 300 K showing the ^{13}C–^{15}N coupling pattern. The spectrum was acquired by using a spectral width of 1850 Hz, 32k data points (digital resolution 0.1 Hz per data point), 5 μs pulse length (30°) and a delay of 10 s between pulses. (*b*) IR stretching absorption of the –CN group in an equimolar mixture of **2a** and **2b**.

labelled on the amino group ($^{15}NH_2CN$). The inverse gate-decoupled ^{15}N NMR spectrum of this compound actually revealed two signals of equal intensity at −213.5 and −384.2 ppm ($^1J_{NH}$ = 88 Hz) from neat nitromethane in keeping with a nitrile structure.[11] These data evidence a complete dispersion of ^{15}N between the two nitrogen atoms that could be due to the hypothetical $H_2NCN \rightleftharpoons NCNH_2$ tautomerism.[11]

By the reaction reported in Scheme 1, a mixture of two labelled species, **2a** and **2b**, in a 1:1 ratio, was obtained. The equal distribution of the marked nitrogen in the cyanamide used prevents any sound speculation on the mechanism of this reaction.

The ^{15}N chemical shifts are reported in Table 1. The availability of the selectively-enriched N(2) and N(3) nitrogen atoms allows a straightforward assignment of the resonance at −26.0 ppm to N(1) while the resonances at −112.7 and −126.2 ppm are assigned to N(2) and N(3), respectively, on the basis of the typical chemical shift for the –CN group[12] and of the broader bandwidth of the signal at −112.7 ppm due to the scalar coupling interaction between $^{15}N(2)$ and $^{14}N(1)$.*

The ^{13}C spectrum (see Table 1), shows four signals in the aromatic region that could be easily assigned on the basis of the hydrogen-coupled spectrum. The chemical shift values of the ring carbons are in keeping with the electron-withdrawing properties of the N(O)=N–CN function (σ_m = 0.78, σ_p = 0.89),[7] which are similar to those of the nitro group (σ_m = 0.74, σ_p = 0.78).[13]

The resonance centred at 110.8 ppm, relative to the nitrile carbon, exhibits the expected scalar coupling constants for ^{13}C–$^{15}N(2)$ (J^1 = 3.7 Hz) and ^{13}C–$^{15}N(3)$ (J^1 = 15.6 Hz) in the two isotopomers [Fig. 1(*a*)].

In this pattern, broader central doublet signals are also evident, owing to the greater scalar coupling interaction of C(5) with ^{14}N in the $=^{15}N$–C≡^{14}N moiety than in $=^{14}N$–C≡^{15}N.

The molecular ion in the mass spectrum occurs at m/z =148 as expected by the presence of one ^{15}N atom per molecule. IR spectra also suggests the presence of isotopic enrichment; in fact, the expected isotopic effect ($\nu^{14}N$ = 1.015 $\nu^{15}N$) doubles the absorption relative to that of the –CN group stretching [Fig. 1(*b*)]. This effect is not clearly detected in the symmetric and asymmetric stretching regions of the N(O)=N group.

Table 1 ^{13}C and ^{15}N chemical shifts of **2**

δ_C(ppm)[a]					δ_N(ppm)[b]		
C(1)	C(2)	C(3)	C(4)	C(5)	N(1)	N(2)	N(3)
144.9	122.8	129.8	135.9	110.8	−26.0	−112.7	−126.2

[a] From TMS. [b] From CH_3NO_2.

Table 2 ^{13}C and ^{15}N spin–lattice relaxation times, T_1, and NOE factors, η of **2**

	C(1)	C(2)	C(3)	C(4)	C(5)	N(1)	N(2)	N(3)
T_1[a]/s	—	8.6	8.6	3.8	17.4	—	97.2[b]	124.2[b]
η	—	1.5	1.5	1.7	0	—	—	—

[a] Measured at 9.4 T. [b] Values measured in the equimolar mixture of **2a** and **2b**.

NMR Relaxation Studies

Besides chemical shifts and coupling constants, the elucidation of structural and dynamic features of molecules in solution is supported by measurements of spin–lattice relaxation times (T_1). Since several mechanisms contribute to T_1, the separation and evaluation of the different contributions allow important parameters, such as bond distances, chemical-shift anisotropies and correlation times (τ_c) to be obtained.

(*a*) *Molecular Correlation Time.*—The longitudinal ^{13}C spin–lattice relaxation times (T_1) and NOE factors, measured at 300 K and 100.5 MHz, are reported in Table 2. The NOEs found for the protonated ring carbons indicate that their relaxation is mainly affected by dipolar interaction with the directly attached proton, with appreciable contribution from the dissolved oxygen. Furthermore, the non-equivalence of their T_1 values provides evidence that the re-orientation of the molecule in solution is anisotropic. The dynamic behaviour in mono-

* On this basis the ^{15}N resonance assignments reported for compounds **1b** 2-phenyldiazene-1-carbonitrile 2-oxide and **1e** 2-pyridyldiazene-1-carbonitrile 2-oxide in ref. 9 must be reconsidered.

Table 3(*a*) ^{13}C and ^{15}N spin–lattice relaxation times, T_1, at different magnetic-field strengths and calculated chemical-shift anisotropies (CSA) of C(5), N(2) and N(3)

	C(5)[a]	N(2)[b]	N(3)[b]
T_1/s (9.4 T)	17.4	97.2	124.2
T_1/s (6.3 T)	26.1	142.9	209.2
T_1/s (4.7 T)	32.0	173.2	281.3
CSA (ppm)	320	330	329

[a] Values measured on **2**. [b] Values measured in the equimolar mixture of **2a** and **2b**.

Table 3(*b*) ^{13}C and ^{15}N chemical shift anisotropies for a few reference compounds

	HCN	CH_3CN	C_6H_5CN
^{13}C CSA (ppm)	282[a]	311[a]	301[a]
^{15}N CSA (ppm)	563[b]	452[c]	336[d]

[a] Ref. 17. [b] Ref. 18. [c] Ref. 19. [d] This work.

substituted benzenes is often simply assumed to consist of an internal rotation of the phenyl group superimposed on the overall isotropic molecular motion.[14,15] In the limit of this approximation, the shorter value of T_1 for the *para* carbon is then accounted for by the fact that the C(4)–H dipolar interaction is not modulated by the preferred phenyl rotation about the C(1)–C(4) axis. It follows that, if we assume the isotropic overall motion model, the molecular re-orientational correlation time (τ_c) can be calculated from the dipolar contribution to the longitudinal relaxation rate (R_1^{DD}) of the *para* carbon, given by eqn. (1).

$$R_1^{DD} = \left(\frac{\gamma_H^2 \gamma_C^2 \hbar^2}{r^6}\right)\tau_c \qquad (1)$$

The dipolar contribution to the measured relaxation rate (R_1^{obs}) has been evaluated from the experimental NOE factor (η^{obs}) according to eqn. (2).[16]

$$\frac{R_1^{DD}}{R_1^{obs}} = \frac{\eta^{obs}}{1.998} \qquad (2)$$

By adopting a standard value of 1.08 Å for the C–H bond length (r), $\tau_c = 6.41 \times 10^{-12}$ s was obtained.

(*b*) *Chemical-shift Anisotropies.*—T_1 for the carbon atom in the nitrile group, C(5), is roughly five times longer than T_1 for the *para* carbon (Table 2). In addition, its magnetic-field dependence [Table 3(*a*)] clearly indicates that the relaxation behaviour is dominated by the chemical-shift anisotropy mechanism, given by eqn. (3)

$$R_1^{CSA} = \frac{2}{15}\gamma^2 H_0^2 (\Delta\sigma)^2 \tau_c \qquad (3)$$

where $\Delta\sigma$ represents the chemical-shift anisotropy. From eqn. (3) and the data reported in Table 3(*a*), reliable values for the product $\Delta\sigma\tau_c$ are obtained. A value of 320 ppm for the ^{13}C chemical-shift anisotropy is then calculated by adopting the τ_c value previously obtained for the *para* carbon [Table 3(*a*)]. ^{13}C CSA values reported in the literature for the –CN group in HCN, CH_3CN and C_6H_5CN [Table 3(*b*)] indicate that this parameter, in a limited series of similar compounds, is quite insensitive to the chemical nature of the substituent. The close similarity between the calculated $\Delta\sigma$ values and those reported in Table 3(*b*), then validates the approximation introduced by assuming an isotropic overall tumbling model.

In addition, the value found confirms the low importance of mesomeric forms involving the nitrile group, as evidenced by the chemical shift and the measured dipole moment[8] of the ONN–CN function. In Table 3 are also reported the ^{15}N T_1 and CSA values obtained following the same procedure. The same CSA value of *ca.* 300 ppm is obtained for both N(2) and N(3). To our knowledge only few data are reported in the literature for the ^{15}N $\Delta\sigma$ of the nitrile group[20] [Table 3(*b*)], showing a strong dependence of this parameter on the chemical nature of the substituent (*ca.* 100 ppm on going from HCN to CH_3CN).

Following the procedure described above, we then measured the ^{15}N CSA in C_6H_5CN, whose value (336 ppm) is very similar to those of N(3). The range of variability of ^{15}N CSAs appears then to be quite large and it may be considered a sensitive probe of the electronic structure of the molecule.

(*c*) C–N *Bond Distances.*—The availability of a mixture of the highly ^{15}N-enriched species **2a** and **2b** should allow, in principle, further structural information to be gained by comparison of the relaxation behaviour of the isotopomers. The longitudinal relaxation rates of C(5) for **2**, **2a** and **2b** are expected to be different because of the different contributions of the dipolar interaction with the nitrogen atoms according to eqn. (4)[16]

$$R_{1CX}^{DD} = \frac{4}{3}\left(\frac{\gamma_C^2 \gamma_X^2 \hbar^2}{r_{CX}^6}\right)(S_X)(S_X + 1)\tau_c \qquad (4)$$

where $X = {}^{14}N$ or ^{15}N. Thus differences in the relaxation rates between **2** and **2a** or **2b** can be expressed as shown in eqn. (5).

$$R_1(\mathbf{2}) - R_1(\mathbf{2a/b}) = R_1^{DD}(^{13}C\text{–}^{14}N) - R_1^{DD}(^{13}C\text{–}^{15}N) = \frac{\gamma_C^2 \hbar^2 (8/3)(\gamma^2\,{}^{14}N - \gamma^2\,{}^{15}N)}{r^6_{C\text{–}N}}\tau_c \qquad (5)$$

If τ_c is known from an independent measurement the parameter r can then be evaluated.

We measured, at 9.4 T, $^{13}C(5)$ T_1 values of 17.37 s for **2**, 17.45 s for **2a** and 17.60 s for **2b**, from which it is possible to calculate bond distances of 1.21 and 1.02 Å for C(5)–N(2) and C(5)–N(3), respectively, by adopting the τ_c value of 6.41×10^{-12} s calculated above. These values represent an average of five determinations with a reproducibility of ± 0.1 s, corresponding to an uncertainty in the calculated distances of *ca.* 0.1 Å. The calculated bond distances are in relatively good agreement with those obtained from X-ray analysis of **1**.[21] The difference between the two sets of values (0.15 and 0.11 Å) cannot be accounted for by the presence of the *para* CO_2H substituent and hence has to be ascribed to the uncertainty associated with the evaluation of small differences between large numbers which, in our case, are in the limits of the experimental error. Nevertheless, although in this case the calculated distances appear to have a limited physical significance, the method described above may have a more general applicability and could be conveniently applied to NMR structural studies.

(*d*) *Rotation of the Phenyl Ring.*—In mono-substituted benzenes, where the anisotropy of the molecular reorientation results in different T_1 values for the protonated carbons of the aromatic ring, the approximation of axially symmetric overall tumbling is often applied.[15,22]

For this simplified model of the phenyl-ring rotation superimposed on the overall motion, the ring *ortho* and *meta* ^{13}C R_1^{DD} values are given by eqn. (6)[23]

Table 4 Molecular motion and phenyl internal rotation parameters for **2**, nitrobenzene and *N,N*-dimethylaniline

Compounds	ρ	$D_o/10^{-10}$ s^{-1}
2	1.824	2.6
$C_6H_5NO_2$	1.875	3.4
$C_6H_5N(CH_3)_2$	1.104	3.7

$$R^{DD}{}_{1o,m} = \frac{\gamma^2{}_H \gamma^2{}_C \hbar^2}{r^6{}_{C-H}} (D_o)^{-1} \frac{1/4(3\cos^2\beta - 1)^2}{6} + \frac{3\sin^2\beta\cos^2\beta}{5 + \rho} + \frac{3/4\sin^4\beta}{2 + 4\rho} \quad (6)$$

where $\rho = D_i/D_o$, and D_o and D_i represent the diffusion coefficients associated with the overall molecular tumbling and the internal motion, respectively, and β is the angle between the C–H vectors and the rotation axis. The ρ parameter then describes the degree of motional anisotropy. The results of the calculations for **2**, nitrobenzene and *N,N*-dimethylaniline are reported in Table 4.

The similarity of the ρ values for 2-phenyldiazene-1-carbonitrile 2-oxide and nitrobenzene is significant, reflecting an analogous behaviour between the steric hindrance and electronic effects of these substitutents.

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