An EPR and NMR Study of some Tetramethylisoindolin-2-yloxyl Free Radicals

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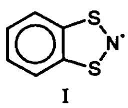
Isoindolin-2-yloxyls are stable free radicals whose potential as spin probes/labels has not yet been exploited. We have prepared 1,1,3,3-tetramethylisoindoline, its 5-nitro derivative (paving the way to adding prosthetic groups) and a version with fully deuteriated methyl groups. These isoindolines have been oxidized to give the corresponding free radicals. It was found that the isotropic EPR spectra of the radicals have linewidths much smaller than those of the TEMPO family of radicals. The parent radical has a spectrum characterized by hyperfine interactions from nitrogen and from protons of the methyl groups. By measuring the NMR chemical shift differences between the isoindolines and the isoindolinyloxyls, values and signs of hyperfine splittings have been determined for the aromatic protons (too small to measure even in well resolved EPR spectra) and for some of the carbon-13 nuclei (at natural abundance). An important advantage of the NMR method is that assignments can be made with near certainty. This advantage has been taken further by obtaining ^1H–^{13}C chemical shift correlation spectra of the free radicals.

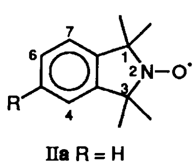
For spin probe measurements, accurate values of the anisotropic g-factors and nitrogen hyperfine interactions are needed: these have been derived from the EPR powder spectrum of the deuteriated radical.

There are few reports on aminoxyl radicals containing a fused aromatic moiety. It is known that such a moiety confers increased stability on similar free radicals as has been shown for 1,3,2-dithiazol-2-yl radicals, for example, benzo-1,3,2-dithiazol-2-yl **I**.[1]



I

Tetraalkylisoindolin-2-yloxyl **II** radicals have been shown [2–5] to be more useful as radical scavengers than the more accessible aminoxyl radicals such as TEMPO.



IIa R = H
IIb R = NO_2

The magnetic resonance work done previously on isoindolinyloxyl radicals is sparse,[6,7] it was done some time ago and it was concerned only with the nitrogen and proton isotropic hyperfine interactions of the 1,1,3,3-tetraethyl derivatives. Because of their stability, isoindolinyloxyl radicals have considerable potential as spin probes hence we have undertaken a comprehensive magnetic resonance study of compounds **IIa** and **IIb** and of the tetra(perdeuteriomethyl) derivative of **IIa**. EPR measurements enable the isotropic hyperfine interactions of the nitrogen nucleus and the nuclei in the methyl groups to be determined: they also allow the anisotropic g-factors and hyperfine interactions of the nitrogen nucleus to be evaluated. NMR spectroscopy has been used to determine the isotropic hyperfine interactions of aromatic protons and of carbon-13 nuclei. The proton coupling constants are too small to measure by ENDOR techniques while the low natural abundance of carbon-13 and the small isotropic hyperfine interactions compound this difficulty.[8] The hyperfine interactions reported in this paper contribute to the larger database required for quantum mechanical calculations.

Experimental

Materials.—We have used the convenient synthesis of 1,1,3,3-tetramethylisoindolin-2-yloxyl described by Griffiths and coworkers.[9] In their method, phthalic anhydride is converted into N-benzylphthalimide which is then treated with methylmagnesium iodide to give N-benzyl-1,1,3,3-tetramethylisoindoline; the latter is hydrogenated to give 1,1,3,3-tetramethylisoindoline which can be oxidized with hydrogen peroxide to give the radical **IIa**. The tetra(perdeuteriomethyl) version of **IIa** can be prepared by using [$^2H_3$]methylmagnesium iodide (Aldrich).

The preparation of compound **IIb** can be carried out simply by nitrating **IIa**. Conc. H_2SO_4 (2 cm^3) was added dropwise to 1,1,3,3-tetramethylisoindolin-2-yloxyl (0.2 g, 1 mmol) cooled in an ice–water bath, forming a dark-brown solution which was then warmed to 60 °C for 15 min and then cooled to 0 °C. Conc. HNO_3 (0.5 cm^3, 2.7 mmol) was added dropwise to the reaction mixture. When reaction appeared complete the solution was heated on a steam bath for 10 min, the colour turning to red-orange. After cooling to room temp., the solution was neutralized by addition to ice-cooled (10%) aqueous sodium hydroxide. The reaction mixture was extracted with diethyl ether and the extracts dried over Na_2SO_4. Red-orange crystals were obtained by recrystallization of the crude material from ethanol (m.p. 160–162 °C) (Found: C, 61.6; H, 6.45; N, 11.9. Calc. for $C_{12}H_{15}N_2O_3$: C, 61.26; H, 6.43; N, 11.91%).

1,1,3,3-Tetramethyl-5-nitroisoindoline was required as a reference compound for the NMR determination of hyperfine interactions but it could not be prepared by nitration of the parent isoindoline and hence the following procedure was adopted. 1,1,3,3-Tetramethylisoindoline (2.5 g, 14 mmol) was dissolved in aqueous NaOH (8 cm^3; 10%) then benzoyl chloride (1.8 cm^3, 15 mmol) was added dropwise with stirring. A precipitate was observed and stirring was continued for a further 10 min. The solid N-benzoyl-1,1,3,3-tetramethylisoindoline thus formed was recrystallized from ethanol. The solid derivative (1.1 g, 4 mmol) was dissolved in a mixture of conc. H_2SO_4 (10 cm^3) and HOAc (5 cm^3). To the ice-cooled mixture

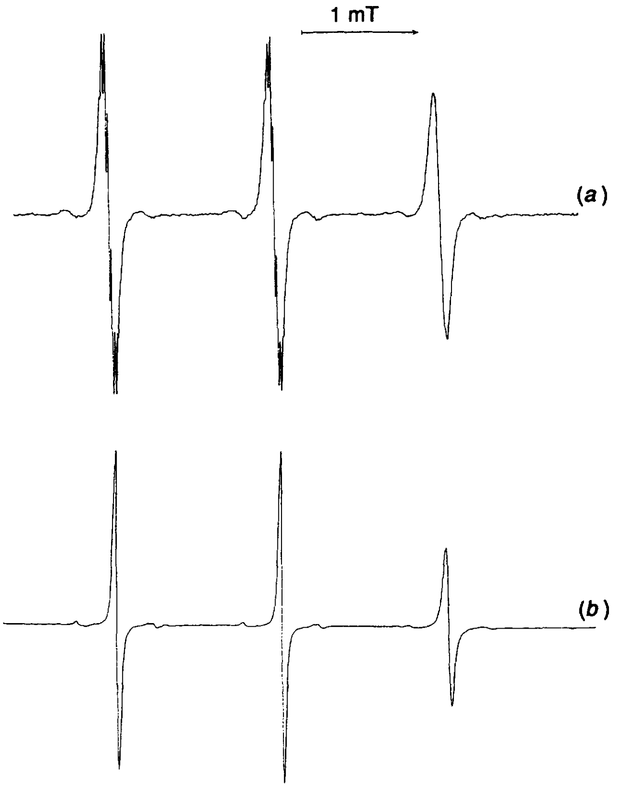


Fig. 1 The first-derivative X-band EPR spectra of (*a*) 1,1,3,3-tetramethylisoindolin-2-yloxyl, **IIa**; (*b*) 1,1,3,3-tetra[$^2H_3$]methyl)isoindolin-2-yloxyl, in toluene at 218 K

Table 1 EPR parameters for 1,1,3,3-tetramethylisoindolin-2-yloxyl **IIa** in toluene

a^N	1.410 ± 0.001 mT[a]
$a^H(CH_3)$	$0.024\,3 \pm 0.000\,4$mT[a]
$a^C(CH_3)$	0.640 ± 0.005 mT[a]
g_{iso}	$2.005\,42 \pm 0.000\,05$[a]
$a^N + A^N_{xx}$	3.382 ± 0.015 mT[b]
$a^N + A^N_{yy}$	0.439 ± 0.015 mT[b]
$a^N + A^N_{zz}$	0.500 ± 0.015 mT[b]
$\langle a^N \rangle$	1.440 mT[b]
g_{xx}	$2.001\,47 \pm 0.000\,05$[b]
g_{yy}	$2.005\,23 \pm 0.000\,05$[b]
g_{zz}	$2.008\,20 \pm 0.000\,10$[b]
$\langle g \rangle$	$2.004\,97$[b]

[a] Values obtained at 218 K. [b] Values obtained at 138 K.

was added dropwise 70% aqueous HNO_3 (0.3 cm^3, 4.3 mmol). After 10 min at 0 °C the mixture was stirred for 3 h at room temperature and then poured into ice–water forming *N*-benzoyl-1,1,3,3-tetramethyl-5-nitroisoindoline as a yellow solid. This product was recrystallized from ethanol, after which it was hydrolysed with 70% w/w aq. H_2SO_4 to give the desired 1,1,3,3-tetramethyl-5-nitroisoindoline which was purified by recrystallization from ethanol, m.p. 109–112 °C (Found: C, 65.55; H, 7.4; N, 12.55. Calc. for $C_{12}H_{16}N_2O_2$: C, 65.43; H, 7.32; N, 12.72%). Of course, yloxyl radicals could also have been prepared by oxidation of this isoindoline.

Equipment.—EPR spectra were obtained with a JEOL RE-1X spectrometer fitted with a DVT2 variable temperature controller and an EF-FC5 gaussmeter. Samples were thoroughly degassed on a vacuum line using several freeze–thaw–pump cycles. A Bruker AC-300E NMR spectrometer was used to obtain the proton and carbon-13 NMR spectra at 300 and 75 MHz respectively: samples were dissolved in deuteriochloroform containing a small amount of tetramethylsilane (TMS) as an internal reference.

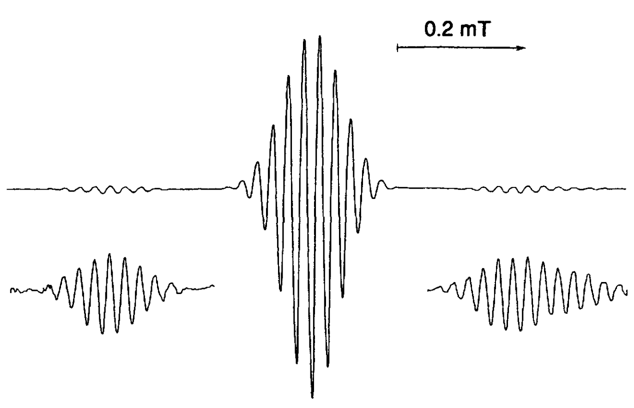


Fig. 2 The low-field part ($m_I^N = +1$) of the second-derivative X-band EPR spectrum of 1,1,3,3-tetramethylisoindolin-2-yloxyl in toluene at 218 K. The carbon-13 satellites are shown at high gain: the high-field group of satellites also includes lines from nitrogen-15 satellites.

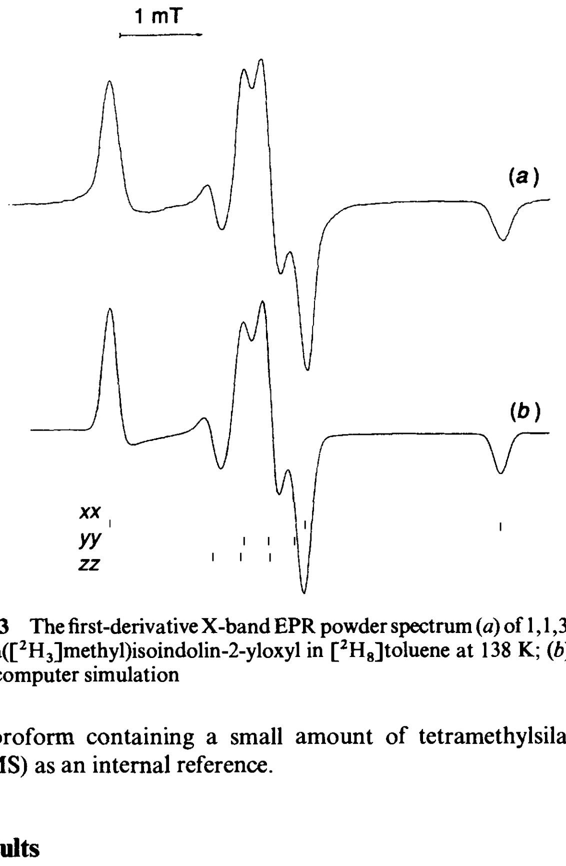


Fig. 3 The first-derivative X-band EPR powder spectrum (*a*) of 1,1,3,3-tetra([$^2H_3$]methyl)isoindolin-2-yloxyl in [$^2H_8$]toluene at 138 K; (*b*) is the computer simulation

Results

EPR Spectroscopy.—In Fig. 1 are shown the isotropic spectra of toluene solutions of compound **IIa** and its deuteriated analogue. Smaller field sweeps of the latter reveal no further fine structure; however, the spectrum of **IIa** shows 13 lines for each of the nitrogen manifolds. The carbon-13 satellite lines are also prominent: a low-field section of the spectrum is shown in Fig. 2. Despite the presence of the methyl group splittings in the spectrum of compound **IIa**, the low-field group of lines shows an overall line width that is about a third of that of TEMPO examined under similar conditions—this provides an important improvement in the signal-to-noise ratio for spin-probe and spin-label applications. If isoindolinyloxyl radicals are to be used for measurements of rotational correlation times, it is necessary to evaluate the anisotropic *g*-factors and the nitrogen hyperfine interactions. For this purpose we have obtained the 'powder' spectrum (see Fig. 3) of the deuteriated version of **IIa**: the parameters derived from the spectrum are listed in Table 1. The isotropic EPR spectrum of compound **IIb** is very similar to

Table 2 Values of a^H and a^C determined from the proton and carbon-13 NMR chemical shifts of compound **IIa** and the corresponding isoindoline

Nucleus	Position	Chemical shifts (ppm) Isoindoline	**IIa**	$a^i/10^{-3}$ mT
^1H	CH_3	1.459	—	—
	5,6	7.133	8.676	−2.14
	4,7	7.245	5.483	+2.45
^{13}C	CH_3	32.003	—	—
	1,3	62.644	—	—
	5,6	121.497	115.35	+2.14
	4,7	127.155	111.86	+5.34
	3a,7a	149.005	43.51	+36.8

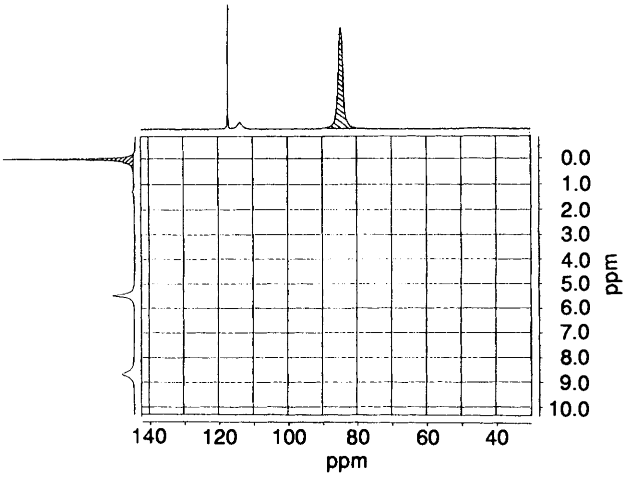


Fig. 4 The 2D proton–carbon-13 NMR chemical shift correlation spectrum of 1,1,3,3-tetramethylisoindolin-2-yloxyl **IIa**, in $CDCl_3$. The shaded lines arise from the proton spectrum of TMS and from the carbon-13 spectrum of $CDCl_3$.

that of **IIa** except that a^N and $a^C(CH_3)$ are slightly smaller having values at 218 K of 1.394 mT and 0.633 mT, respectively. Hatch and Kreilick[10] found in a NMR investigation of TEMPO and some of its derivatives, that $a^C(CH_3)$ has a value of about +0.5 mT and $a^H(CH_3)$ has a value of about −0.023 mT: these values are similar to those we found hence it can be assumed that the signs (not determinable from simple EPR experiments) are the same.

NMR Spectroscopy.—It may be seen from Figs. 1 and 2 that the isotropic EPR spectra of the isoindolin-2-yloxyl radicals have very sharp lines, hence there is no possibility of achieving further resolution in order to observe fine structure originating from the aromatic protons. NMR spectroscopy offers a means not only of measuring these obviously small interactions but also those of the carbon-13 nuclei without resorting to isotopic enrichment. The method is limited to rather small interactions since the NMR linewidth is dependent on 'a' to a power greater than unity. Thus the NMR and EPR methods are complementary. Hyperfine interactions can be measured using NMR spectroscopy by measuring the chemical shift change experienced by a nucleus in a compound when its environment changes from that of a diamagnetic molecule to that of a free radical. We have measured proton and carbon-13 chemical shifts of compounds **IIa** and **IIb** and compared them with the chemical shifts of the corresponding isoindolines. NMR measurements provide an additional benefit in that there is little or no ambiguity in assigning the spectra. An EPR hyperfine coupling constant of a given nucleus i, a^i, can be evaluated with eqn. (1)[11–13] where $\Delta\delta$ is $\delta^i_R - \delta^i_D$, the chemical shift difference (ppm) between the radical and its diamagnetic precursor, γ_e and γ_i are the magnetogyric ratios of the electron and of nucleus i respectively and B_o is the operating field of the NMR spectrometer. Substituting appropriate values into eqn. (1) gives eqns. (2) and (3).

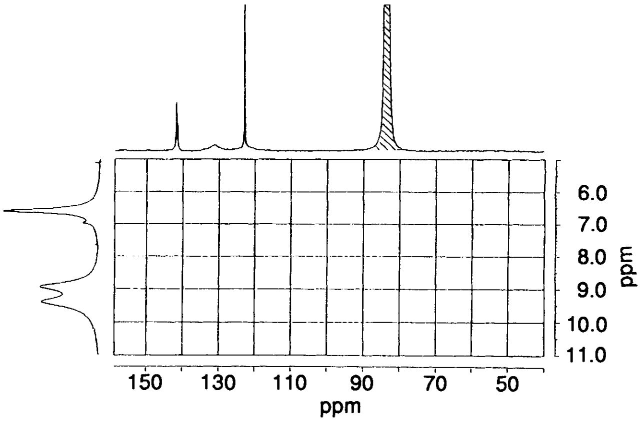


Fig. 5 The 2D proton–carbon-13 NMR chemical shift correlation spectrum 1,1,3,3-tetramethyl-5-nitroisoindolin-2-yloxyl **IIb**, in $CDCl_3$. The shaded line is from the carbon-13 spectrum of $CDCl_3$.

$$a^i = -\Delta\delta/[\gamma_e/\gamma_i)(g\beta B_o/4kT)] \qquad (1)$$

$$a^H = -1.39 \times 10^{-3}\,\Delta\delta\,(\text{mT}) \qquad (2)$$

and

$$a^C = -0.349 \times 10^{-3}\,\Delta\delta\,(\text{mT}) \qquad (3)$$

The 300 MHz proton NMR spectrum of 1,1,3,3-tetramethylisoindoline comprises a single line at 1.459 ppm from the methyl groups and a complex pattern of lines at about 7.2 ppm from the aromatic protons. The latter form an AA′BB′ spin system: the A (protons 5 and 6) and B (protons 4 and 7) chemical shifts are listed in Table 2. The 75 MHz carbon-13 spectrum of the isoindoline is also easy to assign, the data obtained are given in Table 2. The 2D proton–carbon-13 chemical shift correlation for the radical **IIa** is shown in Fig. 4 from which it may be seen that two peaks originating from the aromatic moiety are observed for each nucleus: the low-field carbon line correlates with the high-field proton line. Other correlations are not observed as the line widths are too great. In the carbon-13 spectrum there is a very broad line at 43.51 ppm which we have assigned to the carbons at positions 3a and 7a. From the chemical shift differences and eqns. (2) and (3) we have determined the isotropic hyperfine values given in Table 2. The hyperfine values for the aromatic protons of the 1,1,3,3-tetraethylisoindolin-2-yloxyl have been reported by Sysoeva and co-workers,[6] who used continuous-wave NMR methods, and 2-methylisoindoline as the diamagnetic reference compound. Our results do not agree with theirs: unfortunately, complete spectra were not reproduced and chemical shift data were not quoted in their paper, hence their calculations cannot be checked.

We have obtained similar data for **IIb** and the corresponding isoindoline. The 300 MHz proton spectrum of the isoindoline has two slightly different methyl group resonances at 1.5002 and 1.5150 ppm caused by the asymmetry introduced by the nitro group in position 5. The aromatic protons form an ABX spin system with only the AB (6,7) coupling being large enough to be observed. The assignment of the lines in the carbon-13 NMR spectrum was straightforward; non-equivalence of the 1,1 with the 3,3 methyl groups is observed for these and for the carbons

Table 3 Values of a^H and a^C determined from the proton and carbon-13 NMR chemical shifts of radical **IIb** and the corresponding 5-nitroisoindoline

		Chemical shifts (ppm)		
Nucleus	Position	5-Nitroisoindoline	**IIb**	$a^i/10^{-3}$ mT
^1H[a]	CH_3	1.500 1.515	—	—
	4	7.231 7.261	6.60	+0.90
	7	7.973	8.91	−1.30
	6	8.131 8.159	9.37	−1.70
^{13}C	CH_3	31.699 31.739	—	—
	1,3	62.719 62.958	—	—
	7	117.174	131.27	−4.92
	6	122.215	141.64	−6.78
	4	123.203	122.25	+0.33
	5	147.988	—	—
	8	150.599	—	[b]
	9	155.992	—	[b]

[a] $^3J^H(6,7) = 8.7$ Hz. [b] There is a possible value of +0.04 mT for this carbon.

at positions 1 and 3. From the 2D proton–carbon-13 correlation spectrum shown in Fig. 5 it may be seen that only three lines for radical **IIb** were recorded. In this correlation spectrum, the high-field carbon-13 line correlates with the high-field proton line: because these two lines are the sharpest in the two spectra they must correspond to the smallest hyperfine interaction, hence they have been assigned to position 4. The assignments for positions 6 and 7 are believed to be the most plausible. In the carbon-13 NMR spectrum of radical **IIb** there is evidence for a very broad line at about 54 ppm which could correspond to a hyperfine coupling of about + 0.04 mT for the carbons at positions 7a and 3a. In Table 3 are listed the chemical shifts of **IIb** and of its isoindoline, along with the isotropic hyperfine interactions calculated using eqns. (2) and (3). Once again our values for the aromatic proton hyperfines do not agree either in sign or magnitude with those of Sysoeva and coworkers.[6] Furthermore, these workers arrived at equal a^H values for protons 4 and 7.

Discussion

g-Factors.—The isotropic *g*-factor of the radical **IIa** (see Table 1) has a value somewhat smaller than the values encountered for six- or five-membered ring aminoxyl radicals.[14,15] However, the anisotropic *g*-tensors lying in the plane of the five-membered ring of compound **IIa** have values similar to those determined for five-membered ring aminoxyls in single crystals.[15,16]

Nitrogen Hyperfine Interactions.—The isotropic and anisotropic hyperfine interactions (see Table 1) are as expected for a five-membered ring aminoxyl radical.[16]

Proton Isotropic Coupling Constants.—The protons of the methyl groups have hyperfine interactions very similar in magnitude to those found in the TEMPO family (−0.023 mT).[10] The aromatic proton coupling constants were measured (see Tables 2 and 3) using NMR spectroscopy and it was found that introduction of a nitro group at the 5 position has a marked effect on both their signs and their magnitudes.

Carbon-13 Isotropic Coupling Constants.—The methyl group coupling constant is typical of TEMPO-type aminoxyl radicals which have values of about +0.6 mT.[10] We were not able to determine the carbon-13 coupling constant for the carbons at positions 1 and 3. There are no literature values for the aromatic carbon nuclei. For carbons 5 and 6 of radical **IIa**, the magnitudes are similar to those of the protons at this position, but they have opposite signs as is found for aliphatic ring systems.[10] The carbons at positions 3a and 7a have larger coupling constants.

Again, introduction of a nitro group was seen to have marked effect on the hyperfine interactions. For the nitro-substituted radical, **IIb**, the aromatic proton and carbon coupling constants have the same sign for a given ring position. The coupling constants for the carbon nuclei at positions 6 and 7 are negative (probably due to polarization effects from neighbouring spin densities) whereas they have a positive sign for the parent radical **IIa**. Once more a relatively large positive coupling is indicated for the carbons at positions 3a and 7a.

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