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# Magnetic Resonance Study of 4-Amino-2,2,6,6-tetramethylpiperidine-*N*-oxyl and Its Deuterated Derivatives

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4-Amino-2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMP-$NH_2$) is a spin label suitable for the investigation of polymer systems. The analysis of EPR spectra of spin-labeled samples intended for the determination of the type and correlation time of spin-label rotational reorientation in the system under study requires the knowledge of the proton splitting constants, the hyperfine interactions tensor $A^N$, and the $g$ tensor of this nitroxide. The splitting constants were determined by the NMR method from differences in the shifts of protons in the spectra of nitroxide and of its diagmagnetic analog, 4-amino-2,2,6,6-tetramethylpiperidine. By analyzing the rigid-limit EPR spectrum of 4-acetamino-perdeutero-2,2,6,6-tetramethylpiperidine-*N*-oxyl recorded in $CD_3OD$, we determined the tensors $A^N$ and $g$ in this solvent.

## INTRODUCTION

Lineshapes of the EPR spectra of nitroxides are strongly dependent on the rotational reorientation of these radicals, because of the anisotropy of the hyperfine interaction tensor of the nitrogen atom in the nitroxide group, $A^N$, and the anisotropy of the $g$ tensor (*1*). Stable nitroxide radicals are therefore used in various systems as spin labels or spin probes, giving evidence on their rotational reorientation in the medium under study through their EPR spectra.

Quantitative data on the type and correlation time of rotational reorientation may be obtained by an analysis of the nitroxide EPR spectra, assuming that the $A^N$ and $g$ tensors and the proton splitting constants responsible for the further hyperfine structure of the three main nitrogen lines are known (*1, 2*). In this paper a procedure is described which was employed to obtain these values for 4-amino-2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMP-$NH_2$) (Fig. 1), a suitable spin label, which can be chemically bound on various systems through the functional amine group (*3–6*).

Because of their insufficient resolution, the proton splitting constants of TEMP-$NH_2$ cannot be determined by direct analysis of the EPR spectra. We therefore determined them by the NMR method (*7*), using differences in the shifts of the respective protons of TEMP-$NH_2$ and of its diamagnetic analog, 4-amino-2,2,6,6-tetramethylpiperidine. The $A^N$ and $g$ tensors for TEMP-$NH_2$ in methanol were determined by analyzing the rigid-limit EPR spectrum of the deuterated analog of this nitroxide in methanol-$d_4$. Complete deuteration on the piperidine ring and the



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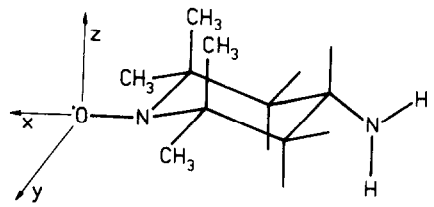


FIG. 1. Structure and molecular axes of TEMP-$NH_2$.

use of a deuterated solvent are necessary, because the presence of proton magnetic moments leads to the line broadening of the rigid-limit EPR spectrum.

## EXPERIMENTAL

### *Preparation of Compounds*

The preparation of 4-amino-2,2,6,6-tetramethylpiperidine and 4-amino-2,2,6,6-tetramethylpiperidine-*N*-oxyl was described earlier (*3*). The preparation of 4-amino-perdeutero-2,2,6,6-tetramethylpiperidine-*N*-oxyl (PD-TEMP-$NH_2$) and of its 4-acetylamino-, 4-glycylamino-, and 4-glycylglycylamino derivatives (Fig. 2) is described below. The preparation of the latter two derivatives is mentioned in this connection, because they were used in the spin labeling of some copolymers (*4*).

Perdeuteroacetone (*8*) and perdeuteroammonia (*9*) were prepared according to the literature. 1H-Perdeutero-2,2,6,6-tetramethyl-II-piperidone (*10*) was prepared according to Ref. (*11*).

4-Hydroximino-1H-perdeutero-2,2,6,6-tetramethylpiperidine: 12 g (0.07 mol) of 1H-perdeutero-2,2,6,6-tetramethyl-4-piperidone, 11 g (0.016 mol) of hydroxylamine hydrochloride, 8 g NaOH (0.2 mol), and 30 ml of deuterium oxide were stirred at room temperature for 60 min. Crystals thus obtained were washed with 5 ml of deuterium oxide and crystallized from the mixture ethanol-hexane (mp, 148–51°C; yield, 10.5 g (80%)).

4-Acetamino-1H-perdeutero-2,2,6,6-tetramethylpiperidine: 8 g (43 mmol) of 4-hydroximino-1H-perdeutero-2,2,6,6-tetramethylpiperidine was dissolved in 300 ml

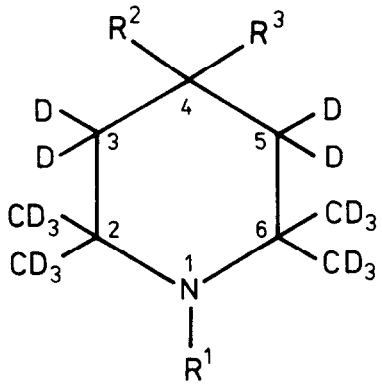


FIG. 2. Structure of the prepared derivatives of perdeutero-2,2,6,6-tetramethylpiperidine (cf. Table 1).

of acetanhydride and shaken at 25°C for 12 hr at 0.04 MPa with 0.8 g of platinum dioxide. Acetanhydride was removed by distillation at reduced pressure, the reaction mixture was alkalized with a 5% NaOH solution, and the crystalline product thus obtained was recrystallized from the mixture ethyl acetate–hexane (mp, 123–5°C; yield, 6.4 g (69.5%)).

4-Acetamino-perdeutero-2,2,6,6-tetramethylpiperidine-*N*-oxyl was prepared by oxidation with hydrogen peroxide (*12*); hydrolysis to 4-amino-perdeutero-2,2,6,6-tetramethylpiperidine-*N*-oxyl was carried out with 15% KOH.

4-Chloroacetamino-perdeutero-2,2,6,6-tetramethylpiperidine-*N*-oxyl: 0.3 mmol (0.54 g) of 4-amino-perdeutero-2,2,6,6-tetramethylpiperidine-*N*-oxyl was dissolved in 50 ml of methylene chloride, cooled to −5°C, and 0.4 mmol (0.4 g) of triethylamine and 0.4 mmol (0.5 g) of chloroacetyl chloride was added to the solution. After 2 hr the reaction mixture was extracted with a solution of 5% HCl and water. The product was crystallized from hexane (mp, 85–6°C; yield, 0.51 g (66%)).

4-Glycylamino-perdeutero-2,2,6,6-tetramethylpiperidine-*N*-oxyl: 0.2 mmol (0.5 g) of 4-chloroacetylamino-perdeutero-2,2,6,6-tetramethylpiperidine-*N*-oxyl was dissolved in 20 ml of methanol saturated with ammonia. The reaction mixture was evaporated after 5 days. The product was crystallized from ethyl acetate with hexane (mp, 96–7°C; yield, 0.4 g (70%)).

4-Chloroacetylglycylamino-perdeutero-2,2,6,6-tetramethylpiperidine-*N*-oxyl was prepared by employing a similar procedure and aminolyzed to 4-glycylglycylamino-perdeutero-2,2,6,6-tetramethylpiperidine-*N*-oxyl. The physical constants of all the derivatives thus obtained are given in Table 1. The molecular masses determined by mass spectroscopy differed by less than 0.1% from the theoretical data in Table 1.

### *Measurements of EPR Spectra*

EPR spectra were recorded with a JES-PE-3X JEOL spectrometer provided with an EC-100 computer. The temperature in the cavity was stabilized with a JES-VT-

TABLE 1

PHYSICAL CONSTANTS OF DERIVATIVES OF PERDEUTERO-2,2,6,6-TETRAMETHYLPIPERIDINE[a]

| $R^1$ | $R^2$ | $R^3$ | mp (°C) | Yield (%) | Molecular formula | Molecular mass |
|---|---|---|---|---|---|---|
| H | O= | | 34–6 | 24 | $C_9HD_{16}NO$ | 171.11 |
| H | HON= | | 148–51 | 80 | $C_9H_2D_{16}N_2O$ | 186.13 |
| H | AcNH–[b] | D | 123–5 | 69.5 | $C_{11}H_5D_{17}N_2O$ | 215.11 |
| O· | AcNH– | D | 146–8 | 60 | $C_{11}H_4D_{17}N_2O_2$ | 230.13 |
| O· | $NH_2$– | D | 35–7 | 49 | $C_9H_2D_{17}N_2O$ | 188.13 |
| O· | ClAcNH– | D | 85–6 | 65 | $C_{11}H_3D_{17}N_2O_2Cl$ | 264.73 |
| O· | H–Gly–NH– | D | 96–7 | 70 | $C_{11}H_4D_{17}N_3O_2$ | 245.32 |
| O· | ClAc–Gly–NH– | D | 145–6 | 71 | $C_{13}H_6D_{17}N_3O_3Cl$ | 321.56 |
| O· | H–Gly–Gly–NH– | D | 196–8 | 79 | $C_{13}H_8D_{17}N_4O_3$ | 302.24 |

[a] See Fig. 2.

[b] Ac = $CH_3$–CO–, ClAc = $ClCH_2$–CO–, Gly = –NH–$CH_2$–CO–.

3A temperature controller and measured with a platinum resistance thermometer. The magnetic field was calibrated with a JES-FC3 NMR marker. The measurements were performed using a microwave power of 1 mW and with a magnetic modulation of 100 kHz. Nitroxide solutions used in the experiments had been deoxygenated by bubbling with nitrogen and were measured in an LC-02 quartz aqueous solution sample tube (active volume, 0.05 ml) under the nitrogen atmosphere. The nitroxide concentration in solutions was of the order of $1 \times 10^{-4}$ *M*.

*NMR Measurements*

NMR spectra were measured on the JEOL PS-100 spectrometer in the field-sweep mode. For the measurement of TEMP-$NH_2$, the 270-ppm sweep width with modulation 8 kHz was used. As the spectra of the free radical were best resolved for the neat substance, both TEMP-$NH_2$ and its diamagnetic analog, 4-amino-2,2,6,6-tetramethylpiperidine, were measured neat at 333 K. Chemical shifts were referred to hexamethyldisiloxane (HMDS) used as an internal standard.

## RESULTS AND DISCUSSION

Shifts $\delta_{para}$ of the individual types of protons determined from the NMR spectrum of TEMP-$NH_2$ (Fig. 3) and shifts $\delta_{dia}$ determined from the spectrum of 4-amino-2,2,6,6-tetramethylpiperidine are given in Table 2. Various peaks in the spectrum of TEMP-$NH_2$ were assigned by comparison with the spectra of similar substances (*7*). The splitting constants calculated from the shift differences using the relation (*7*)

$$a^{H}[G] = -0.04255 \frac{\Delta H\,[Hz] \times T[K]}{H_0\,[G] \times g_e^2} = -1.54 \times 10^{-4} \times \Delta H\,[Hz], \qquad [1]$$

in which $\Delta H = \delta_{para} - \delta_{dia}$, $T$ is the absolute temperature, $H_0$ is the magnetic induction at which NMR spectra were recorded, and $g_e$ is the $g$ factor of the nitroxide studied, are in good fit with values published for similar nitroxides (*7*).

Figure 4 shows the central line of the EPR spectrum of TEMP-$NH_2$ in methanol at 253 K, in which an only partly resolved proton hyperfine structure can be seen. At the same time, Fig. 4 shows a simulated spectral line calculated assuming the Lorentzian lineshape with first-derivative peak-to-peak linewidth $\Delta H_{pp} = 0.22$ G and using proton splitting constants given in Table 2. The good fit between lineshapes of both lines confirms results obtained by the NMR method.

Figure 5 shows the rigid-limit EPR spectrum of 4-acetylamino-perdeutero-2,2,6,6-tetramethylpiperidine-*N*-oxyl recorded at 113 K in $CD_3OD$. This derivative was employed instead of PD-TEMP-$NH_2$ because it can be obtained very pure in the crystalline form. PD–TEMP-$NH_2$ yields a spectrum which is virtually identical. It may be assumed, moreover, that the substitution of the proton of the amine group in position 4 of PD-TEMP-$NH_2$ with the $CH_3$–CO– group does not affect to any essential degree the $g$ factor of nitroxide and the hyperfine interaction tensor of the nitrogen atom in the nitroxide group in position 1, $A^N$. The rigid-limit spectrum was

TABLE 2

CHEMICAL SHIFTS AND SPLITTING CONSTANTS

| Group | Position | $\delta^{a,b}_{para}$ (Hz) | $\delta^{a,c}_{dia}$ (Hz) | $\Delta H^d$ (Hz) | $a^{H^e}$ (G) |
|---|---|---|---|---|---|
| $CH_3$ | 2,6 | 200[f] | −98[f] | 300[f] | −0.047 ± 0.001[f] |
| | | 2900[f] | −107[f] | 3000[f] | −0.462 ± 0.001[f] |
| H | 3,5 | 1850[f] | −162[g] (eq) | 1950 ± 50[f] | −0.30 ± 0.01[f] |
| | | 3050[f] | −73[g] (ax) | 3150 ± 50[f] | −0.49 ± 0.01[f] |
| H | 4 | −900 | −295[g] (ax) | −600 | +0.093 ± 0.001 |

[a] Chemical shifts are referred to hexamethyldisiloxane (HMDS).

[b] Chemical shifts of TDMP-$NH_2$ protons.

[c] Chemical shifts of 4-amino-2,2,6,6-tetramethylpiperidine protons.

[d] $\Delta H = \delta_{para} - \delta_{dia}$.

[e] The $a^H$ were calculated from $\Delta H$ using Eq. [1].

[f] The shifts cannot be assigned to equatorial and axial methyl groups or protons; in the calculation of splitting constants, both possible combinations were always considered, while differences which are pronounced only for protons in positions 3,5 were included into the experimental error.

[g] The $\delta_{dia}$ were assigned to equatorial and axial protons on the basis of the $^1H$ spin-coupling pattern ($^2J_{gem}(3^{eq}, 3_{ax}) = {}^2J_{gem}(5^{eq}, 5^{ax}) = 13$ Hz, $^3J(3_{ax}, 4_{ax}) = 12$ Hz, $^3J(3_{eq}, 4_{ax}) = 4$ Hz).

analyzed by employing the simulation procedure (*2*), assuming Lorentzian lineshape and orientation-dependent linewidth $X = \alpha + \beta \cos^2 \theta$, where $\theta$ is a polar angle between the molecular $z$ axis of nitroxide (Fig. 1) and the direction of the external

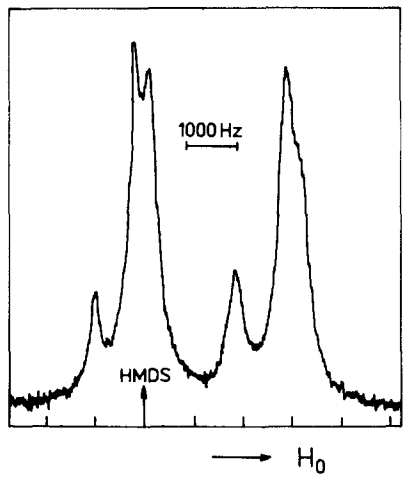


FIG. 3. NMR spectrum of TEMP-$NH_2$ in substance at 333 K.

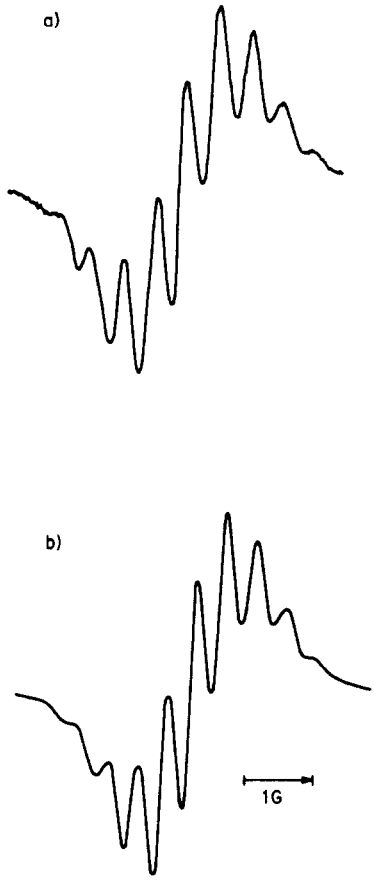


FIG. 4. The central component of the nitrogen splitting of the EPR spectrum of TEMP-$NH_2$ in methanol at 253 K (a) and the simulated one (b) calculated assuming Lorentzian lineshape, first-derivative peak-to-peak linewidth $\Delta H_{pp} = 0.22$ G, and proton splitting constants with values given in Table 2.

TABLE 3

MAGNETIC PARAMETERS[a] OF 4-ACETAMINO-PERDEUTERO-2,2,6,6-TETRAMETHYL-PIPERIDINE-*N*-OXYL

| $A_x$ (G) | $A_y$ (G) | $A_z$ (G) | $\langle A \rangle^b$ (G) | $g_x$ | $g_y$ | $g_z$ | $\langle g \rangle^c$ |
|---|---|---|---|---|---|---|---|
| 7.2 | 5.2 | 35.9 | 16.0 | 2.0090 | 2.0061 | 2.0022 | 2.0058 |

[a] Magnetic parameters were determined by an analysis of the rigid-limit EPR spectrum of the given nitroxide in $CD_3OD$ at 113 K (Fig. 5).

[b] $\langle A \rangle = \frac{1}{3}(A_x + A_y + A_z)$.

[c] $\langle g \rangle = \frac{1}{3}(g_x + g_y + g_z)$.

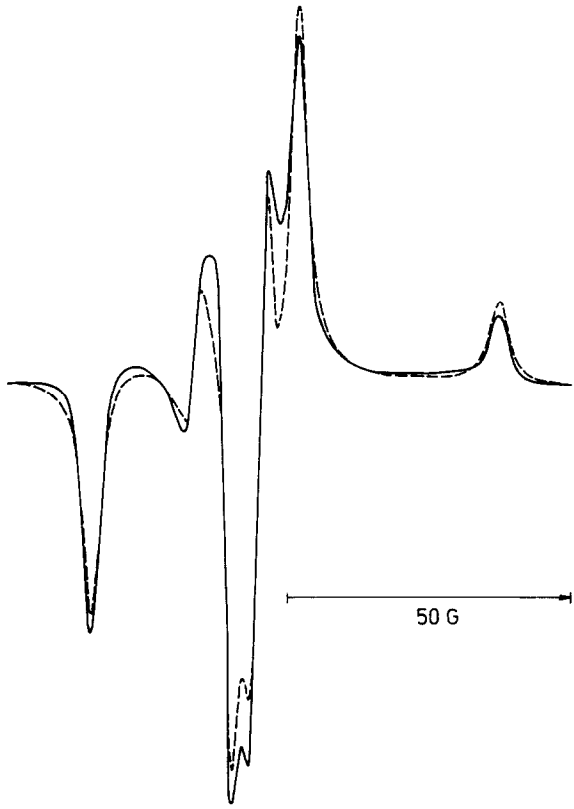


FIG. 5. Rigid-limit spectra (——, experimental; – – –, simulated) of 4-acetamino-perdeutero-2,2,6,6-tetramethylpiperidine-*N*-oxyl in $CD_3OD$ at 113 K.

magnetic field. The best fit between the experimental and simulated spectrum (Fig. 5) was obtained for magnetic parameters given in Table 3 and for $\alpha = 1.55$ G and $\beta = 0.11$ G.

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