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# Preparation of Perdeuteriated 2,5-Di-*tert*-butyl-3,4-di(methoxycarbonyl)pyrroloxyl: a Stable Nitroxide Free Radical with Remarkably Narrow Intrinsic EPR Linewidth

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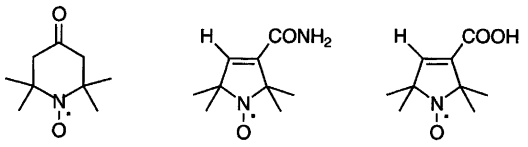
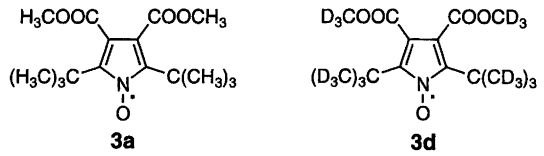
The preparation and EPR spectral properties of a series of deuteriated nitroxides derived from 2,5-di-*tert*-butyl-3,4-dimethoxycarbonylpyrroloxyl is presented. The nitroxides were deuteriated in the methyl ester moiety by means of transesterification with methanol-$d_4$ and in the *tert*-butyl groups via a multistep sequence starting from acetone-$d_6$. The EPR spectra were recorded after treatment of the corresponding hydroxylamines with NiOOH in benzene, and the linewidths compared with those of non-deuteriated and perdeuteriated commercial nitroxides. The fully deuteriated nitroxide **3d** was found to have the narrowest linewidth so far recorded for a nitroxide, 11.3 μT, and a nitrogen coupling constant of 0.44 mT (in benzene).

With the rapid growth of interest in EPR imaging and related techniques,[1,2] a number of nitroxides, including deuteriated ones, have been prepared in the search for useful contrast agents. Examples of nitroxides frequently used for imaging purposes are Tempone (4-oxo-2,2,6,6-tetramethylpiperidin-1-oxyl, **1**)[2,3] and CTPO (3-carbamoyl-2,2,5,5-tetramethylpyrrolin-1-yloxyl, **2a**).[4] These radicals have, in common with most other nitroxides, one major disadvantage for EPR imaging purposes, namely their broad principal nitrogen EPR lines, and the need for nitroxides with decreased intrinsic linewidths for, e.g., oxygen concentration measurements in connection with EPR imaging, has been repeatedly pointed out.[1]

Deuteriation has on a number of occasions[3–5] been shown to result in a narrowing of the linewidth, but the values reported are very sensitive to the experimental conditions, e.g., the nature of the solvent, residual oxygen concentration, free radical concentration, etc. For a comparison of the linewidths from a set of organic free radical EPR spectra to be meaningful, they need to be recorded under identical conditions, and hence a comparison of literature values is best treated as indicative.




To our knowledge, the lowest value hitherto reported for the linewidth of a nitroxide was presented by one of us for 2,5-di-*tert*-butyl-3,4-diethoxycarbonylpyrroloxyl (the diethyl ester of **3a**) more than 20 years ago.[6] This radical also had a remarkably low value for the nitrogen coupling, $a_N = 0.43$ mT (in benzene). The spin density residing on the nitroxide moiety is only ca. 0.6,[6d] (Fig. 1) illustrating that electron-poor nitroxides exhibit lower values of $a_N$ and narrower EPR lines than 'normal' nitroxides (see Table 2 below).

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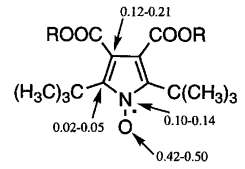



*Fig. 1.* Spin-density distribution in 2,5-dibutyl-3,4-dialkoxycarbonylpyrroloxyl.

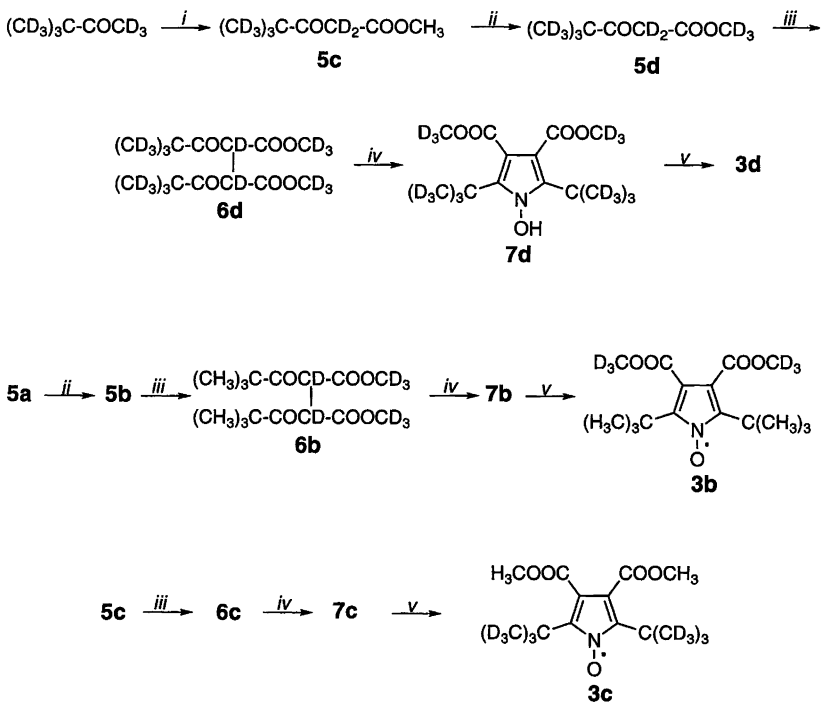
The aim of the present work was to prepare the partially and fully deuteriated analogues of **3a**, i.e., compounds **3b–d**, investigate the effects of deuteriation on their linewidths and compare these values with the values obtained from the non-deuteriated and perdeuteriated nitroxides **1**, **1**-$d_{16}$, **2b** and **2b**-$d_{13}$.

## Results and discussion

*Synthesis.* Compound **3a** was prepared from the methyl ester **5a** as previously described for the ethyl ester,[6a] and **3b** was prepared similarly via transesterification of **5a**. The extreme unwillingness of the methyl ester moiety of **3a**, **6a** and **7a** to transesterify and to undergo conventional hydrolysis obliged us to perform the transesterification step before the dimerization step in the preparation of **3b**. The preparation of **3c** and **3d** started with pinacolization of acetone-$d_6$ followed by pinacol rearrangement and carboxylation to give **5c**, transesterification, dimerization and ring closure with hydroxylamine as summarised in Scheme 1. The very low yields of the hydroxylamines could not be improved either by increasing the temperature or by prolonging the reaction times. The intermediate oximes could be isolated from the reaction mixture (in yields similar to those of the ring-closed products), and these were converted into the hydroxylamines separately under otherwise identical conditions, or simply pooled in the next repetition of the synthesis. The hydrolysis of **7a** was performed with TMSI in $CDCl_3$, giving the dicarboxylic acid **4** in moderate yield.

*EPR measurements.* Summarised in Table 1 are the EPR linewidths for the nitroxides discussed above. All spectra were recorded at high dilution in degassed benzene at 22 °C. Perdeuteriation results in a reduction of the linewidth by a factor of 2.2 for **1** and of 2.5 for **2b** in accordance with earlier observations. For nitroxides **3a–d** deuteriation of the alkyl groups of the ester moiety causes a decrease by a factor of 1.9 (**3c**:**3d**) whereas deuteriation of the *tert*-butyl groups alone has almost no influence (**3a**:**3c**). This is most likely a consequence of the much higher unpaired spin density in the 3-positions than in the 2-positions and hence from only a small contribution to the broadening from coupling of the electron with the protons (or deuterons) of the *tert*-butyl group. In the absence of ENDOR and NMR spectral analysis of the hyperfine splittings no detailed interpretation of the variation of the linewidth with deuteriation can be made, and this will be the subject of a separate study.[7]

The nitrogen coupling constants $a_N$ remained essentially constant at 0.44 mT for the series **3a–d**. It is interesting to note the correlation between $a_N$ and $\Delta H_i$ for a series of nitroxides in which the spin density on the NO




*Scheme 1.* *i*, NaH, $(CH_3)_2OCO$; *ii*, $CD_3OD$, cat. $NaOCD_3$; *iii*, (a) Na–ether, (b) $I_2$; *iv*, $NH_2OH$, $CH_3COOH$; *v*, NiOOH.

*Table 1.* EPR linewidths of non-deuteriated and perdeuteriated nitroxides in benzene at 23 °C.

| Compd. No. | Linewidth/μT |
|---|---|
| **1** | 60.2 |
| **1-$d_{16}$** | 26.6 |
| **2b** | 103 |
| **2b-$d_{13}$** | 22.8 |
| **3a** | 40.7 |
| **3b** | 17.2 |
| **3c** | 21.9 |
| **3d** | 11.3 |

group is varied. In a saturated nitroxide such as **1** $\rho N = \rho O = 0.5$ and the intrinsic linewidth is ca. 30–40 μT. In Frémy's salt, $\rho N + \rho O = 1$ or less if the $SO_3$ groups contribute to some (spin) delocalization and $\Delta H_i = 21$ μT.[3] In the case of diphenyl nitroxide $\rho N = 0.4$ and $\rho O = 0.38$, i.e., the total spin density on the NO-moiety is 0.78,[8] and the experimental spectrum[8a] has been simulated with $\Delta H_i = 16$ μT.[9b] The data are summarized in Table 2, and Fig. 2 shows an almost linear correlation of $(\Delta H_i)^{1/2}$ as a function of $a_N$, even though possible variations of the rotation correlation time are not taken into account. The correlation is not unexpected on theoretical grounds, and a detailed interpretation will be presented later.[7] This demonstrates that a low spin density on the NO-moiety is the key to obtaining small values of $a_N$ and hence narrow intrinsic linewidths for nitroxides.

Finally it is noteworthy that the pyrroloxyl radical **3d** has by far the lowest value yet reported for the linewidth of a nitroxide, and water-soluble derivatives of **3d** could be therefore be interesting as alternatives to **1** and **2** for imaging purposes and relaxation studies.[1b]

## Experimental

*Materials.* 4-Oxo-2,2,6,6-tetramethylpiperidin-1-oxyl, **1** (Jansen, 95%), 4-oxo-2,2,6,6-tetramethylpiperidin-1-oxyl-$d_{16}$, **1**-$d_{16}$ (MSD isotopes, 98 atom% D) and 3-carboxy-2,2,5,5-tetramethyl-3-pyrrolinoxyl-$d_{13}$ **2b**-$d_{13}$ (MSD isotopes, 97.5 atom% D) were used as received. 3-carboxy-2,2,5,5-tetramethyl-3-pyrrolin-1-oxyl, **2b**, was available from earlier work. Methyl 4-methyl-3-oxo-pentanoate (Aldrich, 99%) (**5a**), trimethylsilyl iodide (TMSI, Jansen, 97%), acetone-$d_6$ (Glaser AG, >99.5% D) and methanol-$d_4$ (CIL, >99.8% D) were used as supplied. Pinacolone-$d_{12}$ was prepared from acetone-$d_6$ as described in Ref. 10. for the non-deuteriated compounds. Diethyl ether (Anhydroscan <0.01% $H_2O$) was passed through neutral alumina prior to use. Sodium hydride (Aldrich, 80% suspension in mineral oil) and nickel peroxide (Aldrich) were used as received. All other chemicals were of highest commercial quality available and used as supplied.

*Table 2.* Spin density distributions, nitrogen coupling constants and intrinsic linewidths for some nitroxides.

| | **1** | Frémy's salt | $(C_6H_5)_2NO$ | **3d** |
|---|---|---|---|---|
| $a_N$/mT | 1.5 | 1.2 | 0.97 | 0.44 |
| $\rho N + \rho O$ | 1.0 | 1.0[a] | 0.78 | 0.60 |
| $\Delta H_i$ | 30 | 21 | 16 | ≤11 |
| $(\Delta H_i)^{1/2}$ | 5.5 | 4.6 | 4.0 | 3.3 |

[a] For $\pi O + \rho N \rightarrow \leq 1$.

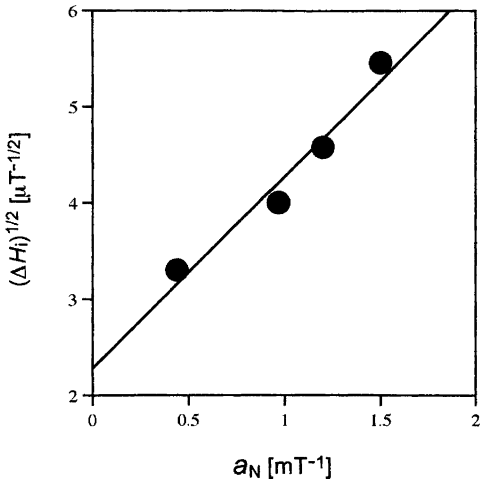



*Fig. 2.* Plot of (intrinsic line width)$^{1/2}$ vs. the nitrogen coupling constant for the nitroxides included in Table 2.

*Instrumentation.* The EPR spectra were recorded with the Upgrade Version ESP 3220-200SH of a Bruker ER-200D SRC instrument at 22 °C. The radical concentration was in the range 0.1–0.2 mM and the modulation amplitude was 1 μT. The microwave power was well below saturation. NMR spectra were recorded on a Varian XL-300 spectrometer. Mass spectra were recorded on a VG Quattro II instrument equipped with ESPC electrospray. GLC analyses were performed on an HP 5830 Ser. II instrument, equipped with a fused-silica column (30 m, 0.25 μm, HP-1701). TLC analyses and column chromatographic separations were performed on Silica Gel 60, using heptane–ether as the eluent.

*Preparation of* **5c**. NaH (22.9 g, 0.77 mol) and dimethyl carbonate (64.1 g, 0.77 mol) were treated with pinacolone-$d_{12}$ (32.4 g, 0.29 mol) as described for the non-deuteriated compound[11] to give **5c** (28.1 g, 0.17 mol, 59%) boiling at 98–100 °C/6 mmHg. $^1$H NMR ($CD_3OD$): δ 3.72 (s, 3 H). $^{13}$C NMR ($CD_3OD$): δ 209.0 (–CO–), 168.8 (–COO–), 51.2 ($-CH_3$), 43.7–42.0 (m, $-CD_2-$), 25.0–23.5 (m, $-CD_3$).

*Preparation of* **5b** *and* **5d**. The methyl-$h_3$ ester (**5a**, **5c**) was dissolved in $CD_3OD$ and treated with 2 mol% $NaOCD_3$. After evaporation the procedure was repeated, and when no protons from the methyl group were discernible by NMR spectroscopy the transesterification was judged to

be complete. The mixture was evaporated, ether was added and evaporated off and the product was used without further purification in the next step.

*Preparation of* **6d**. To a stirred suspension of Na (1.22 g, 53 mmol) in 15 ml of ether under Ar was added **5d** (8.5 g, 49 mmol) in 30 ml of ether over 2 h. After a further 4 h of stirring, a solution of $I_2$ (6.35 g, 25 mmol) in 50 ml of ether was added dropwise over 1 h. The mixture was left overnight and the resulting white suspension was poured into ether–saturated aq. NaCl. The aq. layer was extracted twice with ether and the combined organic layers were dried over $MgSO_4$, evaporated and chromatographed. 4.8 g (14 mmol, 56%) **6d** were collected as a colourless oil, consisting of a mixture of diastereomers and with incomplete deuteriation at the two asymmetric (and acidic) carbons. $^{13}C$ NMR ($CD_3OD$): 209.0 + 208.1 (–CO–), 168.5 + 168.2 (–COO–), 53.7–53.0 (m, $-CD_2-$ + –CHD– + $-CH_2-$), 51.7–50.7 (m, ester–$CD_3$), 25.0–23.5 (m, $-CD_3$). MS ($ESP^+$), *m/z*: 379 (*M* + 39), 363 (*M* + 23). **6a–6c** were similarly prepared from **5a–5c** in 40–60% yield.

*Preparation of* **7d**. $NaOCOCH_3$ (1.78 g, 13.0 mmol), $NH_2OH \cdot x$HCl (0.80 g, 11.5 mmol) in 13 ml $H_2O$ and **6d** (2.80 g, 8.2 mmol) in 35 ml $CH_3COOH$ were mixed and stirred at 65 °C for 72 h. The mixture was cooled and most of the solvent was evaporated off. The residue was poured into ether–aq. $NaCHO_3$ and the aq. layer was extracted with ether. The combined ethereal layers were dried over $Na_2SO_4$ and evaporated and the resulting oil was chromatographed to give 1.6 g of recovered starting material, followed by the oxime (0.025 g, 0.07 mmol, 2.0%) and **7d** (0.065 g, 0.19 mmol, 5.4%) as white crystals, m.p. 156–158 °C. $^1H$ NMR ($CD_3CN$): δ 9.82 (s, 1 H). $^{13}C$ NMR ($CD_3CN$): δ 168.3, 136.5, 109.3, 52.2–50.9 (m, ester-$CD_3$), 33.5, 30.2–28.1 (m, *t*-$CD_3$). MS (ESP) *m/z*: 334 (*M* – 1). Similarly prepared were: **7a** $^1H$ NMR [$(CD_3)_2CO$]: δ 9.80 (s, 1 H), 3.67 (s, 6 H), 1.40 (s, 18 H). MS (ESP) *m/z*: 310 (*M* – 1). **7b**, $^1H$ NMR [$(CD_3)_2CO$]: δ 9.80 (s, 1 H), 1.40 (s, 18 H); $^{13}C$ NMR [$(CD_3)_2CO$]: δ 167.1, 135.3, 108.7, 45.8–44.7 (m, $CD_3$), 33.4, 29.3; MS (ESP) *m/z*: 316 (*M* – 1). **7c**, $^1H$ NMR [$(CD_3)_2CO$]: δ 9.87 (s, 1 H), 3.63 (s, 6 H). $^{13}C$ NMR [$(CD_3)_2CO$]: δ 167.0, 135.5, 108.7, 50.9, 32.3, 29.7–28.2 (m, $-CD_3$). MS (ESP) *m/z*: 328 (*M* – 1).

*Preparation of pyrroloxyl radicals*. To a degassed solution of 2 mg of the hydroxylamine **7a–7d** in 2 ml of benzene were added ca. 10 mg NiOOH. After 5 min the suspension was filtered and the pale green–blue solution was diluted with degassed benzene in order to obtain a solution suitable for EPR spectroscopy. The results are summarised in Table 1.

*Preparation of 2,5-di-*tert*-butyl-*N*-hydroxypyrrole-3,4-dicarboxylic acid (***4***)*. When **7a** was subjected to the transesterification conditions described above, treated with pig liver esterase[12] or subjected to standard alkaline hydrolysis conditions no reaction was observed. **7a** (0.090 g, 0.29 mmol) was dissolved in 10 ml of dry $CDCl_3$ and TMSI (0.240 g, 1.20 mmol) was added. The mixture was heated to 55 °C overnight after which it was diluted with 40 ml of $CH_2Cl_2$ and washed with sat. aq. NaCl, a few drops of aq. $Na_2S_2O_4$ in sat. aq. NaCl and finally sat. aq. NaCl. The organic phase was dried over $Na_2SO_4$ and evaporated, and the remaining solid was dissolved in heptane–ether 9:1, evaporated and triturated with heptane to give the dicarboxylic acid **4** (0.040 g, 0.14 mmol, 49%) as colourless crystals. $^1H$ NMR [$(CD_3)_2CO$]: δ 1.50 (s). $^{13}C$ NMR [$(CD_3)_2CO$]: δ 159.5, 141.0, 108.2, 33.7, 29.2. MS (ESP) *m/z*: 264 (*M* – 19). Upon treatment with NiOOH as described above an EPR signal with a linewidth almost identical with that of **3b** was recorded.

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## References

1. (a) Eaton, G. R., Eaton, S. S. and Ohno, K., Eds. *EPR Imaging and In Vivo EPR*, CRC Press, Boca Raton 1991; (b) Berliner, L. and Reuben, J., Eds. *Spin Labelling; Theory and Applications. Biological Magnetic Resonance, Vol. 8*, Plenum, New York, 1989; (c) Swartz, H. M. *Pure Appl. Chem. 62* (1990) 235.
2. Swartz, H. M. and Glockner, J. F. In: Hoff, A. J., Ed., *Advanced EPR*, Elsevier, Amsterdam 1989, Chap. 21.
3. Chiarelli, R. and Rassat, A. *Tetrahedron 29* (1973) 3639.
4. (a) Halpern, H. J., Spencer, D. P., Van Polen, J., Bowman, M. K., Nalson, A. C., Dowey, E. M. and Teicher, B. A. *Rev. Sci. Instrum. 60* (1989) 1080; (b) Halpern, H. J., Peric, M., Nguyen, T. D. and Spencer, D. P. *J. Magn. Reson. 90* (1990) 40; (c) Halpern, H. J., Peric, M., Yu, C. and Bales, B. L. *J. Magn. Reson. A 103* (1993) 13; (d) Lin, Y. J., Teicher, B. A. and Halpern, H. J. *J. Labelled Comp. Radiopharm. 28* (1990) 621.
5. Bales, B. L. in Ref. 1(b), p. 77.
6. (a) Ramasseul, R. and Rassat, A. *Bull. Soc. Chim. Fr. 72* (1970) 4330; (b) Ramasseul, R. and Rassat, A. *Quantum Aspects of Heterocyclic Compounds in Chemistry and Biochemistry, II*, The Israel Academy of Sciences and Humanities, 1970, pp. 270–275; (c) Cauquis, G., Grossi, P. J., Rassat, A. and Serve, D. *Tetrahedron Lett. 21* (1973) 1863; (d) Grossi, P. J., Marchetti, L., Ramasseul, R. and Rassat, A. *J. Chim. Phys. 74* (1977) 1167.
7. Ardenkjaer-Larsen, J.-H. and Rassat, A. *To be published.*
8. Buchachenko, A. L. *Stable Radicals*. Cosultant Bureau, New York 1965, pp. 129.
9. (a) Lemaire, H., Rassat, A. and Ravet, J. P. *Tetrahedron Lett. 47* (1964) 3507; (b) Lemaire, H. *Thesis*, Grenoble 1966 (Rapport CEA 3119), p. 16.
10. *Org. Synth. Coll. Vol 1*, pp. 459, 462.
11. Swamer, F. W. and Hauser, C. R. *J. Am. Chem. Soc. 72* (1950) 1356.
12. Ohno, M., Ito, Y., Arita, M., Shibata, T., Adachi, K. and Sawai, H. *Tetrahedron 40* (1984) 145.

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