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Nitroxides: Synthesis and Paramagnetic Properties of an α-Hydroxymethyl Derivative of DOXYL

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Abstract: The synthesis of new but unstable α-(hydroxymethyl)oxazolidin-3-oxyls was achieved after protection by silylation of the hydroxyl groups in order to avoid decomposition in diamagnetic nitrones. Relaxivity studies of (*R,S*)-4-(hydroxymethyl)-2,2,4-trimethyloxazolidin-3-oxyl **1** show no beneficial effect due to the presence of an hydroxymethyl group in the vicinity of the N-O group on the paramagnetic properties of nitroxides.

INTRODUCTION

The synthesis of new nitroxides, carrying an hydroxyl function in the vicinity of the paramagnetic center, is part of a research devoted to the design of contrast enhancing agents for Magnetic Resonance Imaging (MRI).[1-6] Suitable molecules should exhibit high paramagnetic properties and vectorisation potentialities. New nitroxides are also of interest in the context of biological and clinical applications of Electron Spin Resonance Imaging[7,8] and Dynamic Nuclear Polarisation[9,10] techniques.

Bennett et al[11,12] reported fatty acid nitroxides bind to albumin and significantly promote the NMR relaxation of water protons. Such a relaxation enhancement involves water molecules hydrogen bonded around the nitroxyl groups in the aqueous solutions of nitroxide/albumin complexes.[13] The introduction of an hydrophilic moiety in the nitroxide molecule near the paramagnetic N-O group might also increase such interactions with water molecules and result in further enhanced relaxivity.

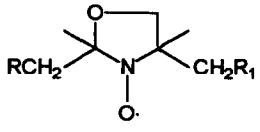
Some α-(hydroxyalkyl)imidazolinoxyl-3-oxides have been mentioned in the litterature.[14] However, attempts to branch an hydrophilic substituent in the close surroundings of the N-O group have often encountered the problem of a poor stability of the desired compounds.[15,16] Nitroxide α-carboxylate salts have been described as stable forms of the unstable free acid derivatives.[15] Deprotection of silyl-protected nitroxyl groups was used to synthesize nitroxides carrying an α-hydroxymethyl group.[16] Unfortunatly, these compounds were also found to be unstable. In this paper, we report a new synthetic approach where the protected function is not the nitroxyl group but the α-hydroxymethyl one. Deprotection is subsequently carried out under mild non acidic conditions. Such a synthetic scheme could be generalized to the synthesis of nitroxide radicals which requires the oxidation of the secondary amine to be performed in the presence of the hydroxymethyl group. The paramagnetic properties of the newly synthesized α-hydroxymethyl nitroxides in solution are evaluated and discussed.

RESULTS AND DISCUSSION

Chemistry

The synthesis of the three racemic α-hydroxymethyl **1** and **2** and α,α'-dihydroxymethyl **3** derivatives of 2,2,4,4-tetramethyloxazolidin-3-oxyl, Figure 1, starts with the classical formation of the five membered heterocycle,[17] as shown in Scheme 1. Ring closure in refluxing toluene for 48 h with paratoluenesulfonic acid as a catalyst was achieved in good yields (62 % and 72 %, respectively) for compounds **9** and **10** with the silyl-protected acetol **7**. Silylation of the hydroxyl group[18,19] was prefered to the protection by a tetrahydrofuranyl ether or an acetate since none of these two protective groups avoids further aldol condensation. The cyclisation of the silyl-protected acetol **7** with 2-amino-2-methyl-1,3-propanediol **4** afforded a mixture **10a,b** of the *cis* and *trans* oxazolidines. Such a mixture was clearly shown by the ^1H NMR spectrum which contains two peaks (δ: 1.32 and 1.5) of different intensities (relative ratio: 1-4) for the $-CH_3$ groups attached to positions 2 and 4 of the oxazolidine. The diastereoisomers were not separated at this stage of the process.

Figure 1

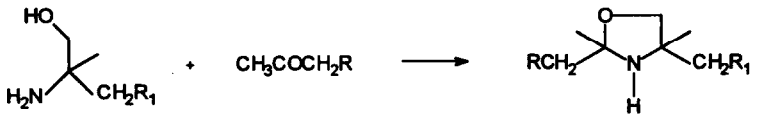


1 R=H R_1=OH

2 R=OH R_1=H

3 R=R_1=OH

Scheme 1



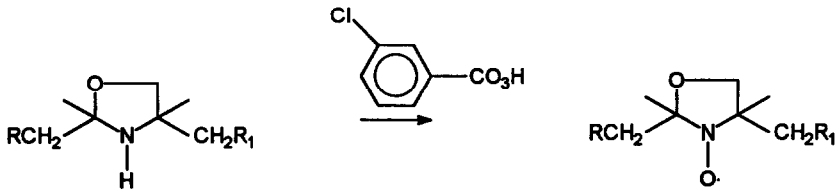
4 R_1=OH **6** R=H **8** R=H, R_1=OH

5 R_1=H **7** R=$OSi(CH_3)_2C(CH_3)_3$ **9** R=$OSi(CH_3)_2C(CH_3)_3$, R_1=H

10a,b R=$OSi(CH_3)_2C(CH_3)_3$, R_1=OH

The hydroxyl groups of compounds **8** and $\mathbf{10_{a,b}}$ were protected in 72 % and 68% yield, respectively, before oxidation of the intracyclic nitrogen atom. Without protection, oxidation of **8** produces a diamagnetic substance identified by ^1H NMR and mass spectroscopy as the corresponding nitrone (see experimental section). This process might involve the oxidation of the hydroxyl compound in an unstable and non-isolable acid which further decomposes in the diamagnetic nitrone. Such a side reaction, wich precludes direct access to nitroxide, has already been observed during Jones oxidation of similar compounds.[16] After silylation of the mixture of the two diastereoisomers $\mathbf{10_a}$ and $\mathbf{10_b}$, a mixture of compounds $\mathbf{12_a}$ and $\mathbf{12_b}$ was obtained. The ^1H NMR spectrum of this mixture exhibits four peaks (δ: 1.32, 1.36, 1.39 and 1.48) of different intensities for the methyl groups on position 2 and 4. Although one of these two oxazolidines was isolated as a cristalline compound, single cristal X-ray experiments did not allow unequivocal determination of the exact configuration because of stability problems.

Scheme 2



11 R=H, R_1=$OSi(CH_3)_2C(CH_3)_3$ **13**

9 R=$OSi(CH_3)_2C(CH_3)_3$, R_1=H **14**

12a,b R=R_1=$OSi(CH_3)_2C(CH_3)_3$ **15a,b**

Oxidation of the silyl-protected oxazolidines **11** and **9** with m-chloroperbenzoic acid in methylene chloride afforded the stable nitroxide radicals **13** and **14** in 18 % and 24 % yield, respectively, Scheme 2. The mixture of compounds $\mathbf{12_a}$ and $\mathbf{12_b}$ gave nitroxides $\mathbf{15_a}$ and $\mathbf{15_b}$ which were separated by silica gel column chromatography. In this case, respective yields of the two racemic *cis* and *trans* compounds were dramatically low, i.e. 5.3 % and 2.7 %. Only one of the two silyl-protected diastereoisomers $\mathbf{15_a}$ and $\mathbf{15_b}$ was obtained in quantity large enough to pursue the synthesis of the α,α'-dihydroxymethyl nitroxide derivative.

Since the nitroxide function is unstable in acidic medium,[20-22] the silyl groups were removed by tetrabutylammonium fluoride in tetrahydrofuran.[18] Nevertheless, this specific cleavage needed a final wash with a small amount of NaCl-saturated water. Despite of this last treatment and a consecutive chromatographic separation from the large excess of cleavage reagent, the hydrophilic nitroxides **1** and **2** were obtained in reasonable yields (37 % and 12 %, respectively). Obtention from $\mathbf{15_a}$ of the more hydrophilic and surely more unstable nitroxide $\mathbf{3_a}$ appeared to be much more difficult, as confirmed by a 3 % yield. These three nitroxides could be stored for several days at -4°C without decomposition.

The ESR spectra of compounds **1**, **2** and $\mathbf{3_a}$ in ethanol consisted of characteristic triplets with a_N values between 14.5 G and 15.6 G. Unfortunatly, **2** and $\mathbf{3_a}$ underwent rapid decomposition to diamagnetic products at 25 °C under air. Nitroxide **2** was nevertheless characterized by mass spectrometry and ^{1}H NMR spectroscopy after reduction of the N-O group to N-hydroxylamine (see experimental section).[23] Only nitroxide **1** was stable enough to allow further characterization.

(*R,S*)-4-(hydroxymethyl)-2,2,4-trimethyloxazolidin-3-oxyl **1** was isolated as a pure compound as determined by TLC, microanalytical data and ^{1}H NMR spectroscopy after phenylhydrazine reduction (see experimental section). FAB mass spectrometry experiments in glycerol showed peaks at MH^+ = 161 (1.5 %) and MH_2^+ = 162 (20 %). Such reduction process has already been described for similar compounds.[24,25]

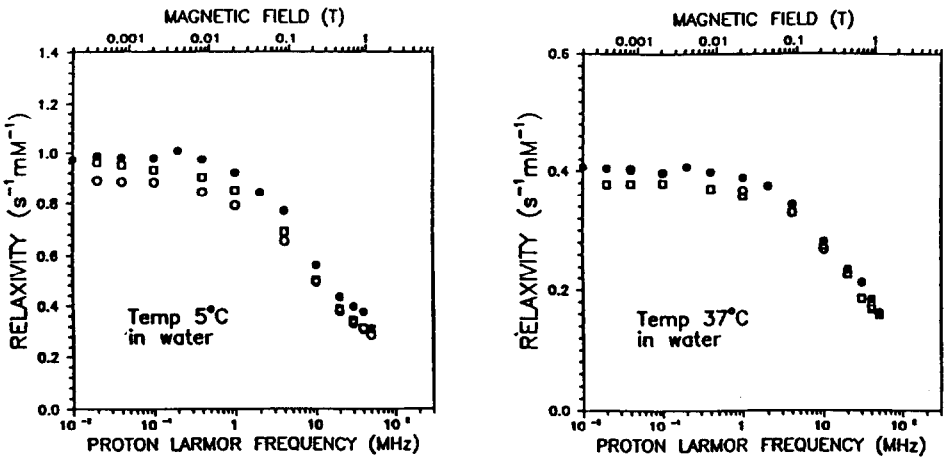
Paramagnetic properties

As a paramagnetic substance, **1** should shorten the magnetic relaxation times of surrounding water hydrogen nuclei. Water proton longitudinal relaxation rates R_1 of an aqueous solution of **1** have thus been measured at 5°C and 37°C over a range of Larmor frequencies extending from 0.01 to 50 MHz. The Nuclear Magnetic Relaxation Dispersion (NMRD) profiles obtained were compared to those of tempol (4-hydroxyl-2,2,6,6-tetramethylpiperidin-1-oxyl) and doxyl (2,2,4,4-tetramethyloxazolidin-3-oxyl) taken as reference compounds.

Relaxivity r_1 represents the increase of the water proton longitudinal relaxation rate induced by one millimole of the paramagnetic species per liter and is expressed in $s^{-1}.mM^{-1}$.

Considering the probable unstability of **1** in solution and owing to the necessity of getting a precise knowledge of the concentration of the paramagnetic structure in order to assess its relaxivity, titration of the compound was performed concomitantly to the recording of the NMRD profiles. This quantitation was achieved by progressive chemical reduction of approximately 10^{-2} M ethanolic solution of nitroxides by known amounts of phenylhydrazine and followed by relaxometry, as reported by Muller et al.[26] This approach showed that the aqueous solution submitted to the relaxometric analysis contained but 17 % of the paramagnetic structure. This result was supported by ESR analysis where peak amplitudes were compared to those recorded on ethanolic solutions of weighed amounts of tempol. These ESR evaluations carried out independently of the relaxometric studies and run on more highly diluted (10^{-4} M) ethanolic solutions indicated 34 % of paramagnetic form. Although nitroxide **1** appears to be pure when isolated as a yellow oil (see experimental part), it undergoes decomposition in solution.

Figure 2a,b: Relaxivity of **1** (○) Compared to the Relaxivities of Tempol (●) and Doxyl (□) at 5 °C and 37 °C, in Water.



Considering the fraction of paramagnetic structure existing in the samples submitted to relaxometric study (17 %), relaxivities were calculated in water at 5 °C and 37 °C (Figure 2a,b). It has to be mentioned that the values recorded at all frequencies were stable during the course of the experiments. The NMRD profiles were compared to those of tempol and doxyl. Interestingly, for this

fraction of **1** with a pure paramagnetic N-O moiety, no major influence of the hydrophilic α-hydroxymethyl group was depicted since relaxation rates profiles of **1**, tempol and doxyl were similar.

In conclusion, we have described a new synthetic method to obtain nitroxide radicals with an hydroxymethyl group in position α. Most derivatives exhibited poor chemical stability. **1** was isolated as a pure compound but proved to undergo decomposition in hydroxylic solvents. It was nevertheless possible to show that the presence of an hydrophylic group, such as an hydroxymethyl, located at close vicinity of the paramagnetic N-O group did not modify the induced relaxivity in spite of the potential modification of the water distribution and dynamics around the paramagnetic center.

EXPERIMENTAL

All reagents were of the finest quality available commercially. 3-chloroperbenzoïc acid was obtained from Aldrich with an analytical purity of 50-60 % and used without further purification. ^1H NMR spectra were recorded in $CDCl_3$ on a Varian 360A or a Bruker AC 250 NMR spectrometer. Chemical shifts are reported in δ units. IR spectra were performed on a Perkin Elmer-983-G infrared spectrometer. ESR spectra were obtained at room temparature and 10 GHz on a Bruker ER 200 D ESR spectrometer. Longitudinal relaxivity profiles have been measured over a magnetic field range extending from 0.0002 Tesla to 1.2 Tesla on an IBM Research Field Cycling Relaxometer.[28] Mass spectrometric determinations were recorded on a Jeol JMS-DX 300 machine. Microanalyses were performed on an 1108 Erba Sciences analyser at the CNRS microanalytical center in Montpellier.

Tertiobutyldimethylsilyloxypropanone (7). Acetol (10 g, 135 mmol) was added dropwise to a stirred solution of tertiobutyldimethylsilyl chloride (22.6 g, 150 mmol) and 1,8-diazabicyclo[5,4,0]undec-7-ene(24.6 g, 162 mmol) in 300 ml of methylene chloride. The reaction was stirred 24 hours at room temperature. The mixture was washed with 100 ml of saturated aqueous sodium chloride, 0.1 M aqueous HCl and saturated aqueous sodium hydrogenocarbonate. After drying with anhydrous sodium sulfate, the organic layer was evaporated and the residue was chromatographied on a silica gel column (methylene chloride-methanol, 98:2) to give the silylated acetol **7** (18 g, 72 %). δ_H ($CDCl_3$) 0.05 (6H, s), 0.9 (9H, s), 2.05 (3H, s), 4.1 (2H, s).

2,2,4-trimethyl-4-(hydroxymethyl)oxazolidine (8): A solution of 2-amino-2-methyl-1,3-propanediol **4** (15.75 g, 150 mmol) and toluene-p-sulfonic acid monohydrate (5mg) in toluene (50 ml) was added dropwise to a solution of acetone (10 g, 172mmol) in toluene (250 ml). The reaction mixture was stirred and refluxed for 48 hours with continuous water removal by means of a Dean - Stark trap. Then the toluene solution was filtered and evaporated to give a yellow oil, which was chromatographied on a silica-gel column with methylene chloride-methanol (95:5) as eluant, to give the corresponding oxazolidine **8** (15.7 g, 72 %) (Found: C, 58.02; H, 10.66; N, 9.73. $C_7H_{15}O_2N$

requires C, 57.89; H, 10.42; N, 9.65); δ_H ($CDCl_3$) 1.4 (3H, s), 1.5 (3H, s), 1.58 (3H, s), 2.8 (1H, broad), 3.45 (2H, s), 3.65 (2H, m).

Direct oxidation of 8: A stirred solution of oxazolidine **8** (1 g, 6.5 mmol) in methylene chloride (12 ml) at -5°C was treated with a solution of 3-chloroperoxybenzoic acid (2.3 g, 13.3 mmol) in methylene chloride (7 ml). Over a 1 h period, the solution became green and a precipate of 3-chlorobenzoic acid had formed. The reaction mixture was filtered, and the filtrate was washed with aqueous 5% sodium carbonate, dried with calcium chloride and evaporated. The residue was chromatographied on a silica gel column (methylene chloride - methanol, 99:1) to give (0.63 g, 75 %) 2,2,4-trimethyl-3,4-dihydro-2*H*-oxazolidine-3-oxide. MS (EI) analysis: m/z 129 (M^+, 26 %), 98 (M^+ - 31, 34 %); IR (neat) 1620 cm^{-1}; δ_H ($CDCl_3$) 1.6 (6H, s), 2.1 (3H, s), 3.8 (2H, m).

2-(Tertiobutyldimethylsilyloxy)methyl-2,4,4-trimethyloxazolidine (9) and 2,4-dimethyl-4-hydroxymethyl-2-(tertiobutyldimethylsilyloxy)methyloxazolidine ($10_{a,b}$) were synthesized from **7** and 2-amino-2-methylpropanol **5** for **9** and **7** and 2-amino-2-methyl-1,3-propanediol **4** for **$10_{a,b}$** using a similar procedure as described for **8**. **9**, yield 61 % (Found: C, 60.01; H, 11.12; N, 5.35. $C_{13}H_{29}O_2NSi$ requires C, 60.19; H, 11.28; N, 5.40); δ_H ($CDCl_3$) 0.05 (6H, s), 0.9 (9H, s), 1.25 (3H, s), 1.3 (3H, s), 1.4 (3H, s), 2.4 (1H, broad), 3.45 (2H, s), 3.5-3.7 (2H, m). **$10_{a,b}$**, yield 68 %, was obtained as a mixture of the two diastereoisomers (Found: C, 56.75; H, 10.67; N, 4.85. $C_{13}H_{29}O_3NSi$ requires C, 56.69; H, 10.62; N, 5.09); δ_H ($CDCl_3$) 0.05 (12H, s), 0.9 (18H, s), 1.32 (6H, s), 1.5 (6H, s), 3.19 (2H, broad), 3.57 (4H, s), 3.65-4 (4H, m).

2,2,4-Trimethyl-4-(tertiobutyldimethylsilyloxy)methyloxazolidine (11). To a stirred solution of tertiobutyldimethylsilyl chloride (0.33 g, 2.22 mmol) and 1,8-diazabicyclo[5,4,0]undec-7-ene (0.37 g, 2.4 mmol) in 4 ml of methylene chloride, was added dropwise a solution of oxazolidine **8** (322 mg, 2.22 mmol). The reaction was stirred for 30 h. at room temperature. The mixture was washed successively with 50 ml of saturated aqueous sodium chloride, 0.1 M aqueous hydrochloric acid and 50 ml of saturated sodium hydrogenocarbonate. After drying with anhydrous sodium sulfate, the organic layer was evaporated. The residue was chromatographied on a silica gel column with methylene chloride-methanol (98:2) as eluant to give **11** (414 mg, 72 %) (Found: C, 59.98; H, 11.17; N, 5.63. $C_{13}H_{29}O_2NSi$ requires C, 60.19; H, 11.28; N, 5.40); δ_H ($CDCl_3$) 0.05 (6H, s), 0.93 (9H, s), 1.2 (3H, s), 1.35 (3H, s), 1.45 (3H, s), 2.52 (1H, broad), 3.4 (2H, s), 3.6 (2H, m).

2,4-Dimethyl-2,4-(ditertiobutyldimethylsilyloxy)methyloxazolidine ($12_{a,b}$) was synthesized from **10a,b** using a similar procedure as described for **11**. Yield 73 % (Found: C, 58.87; H, 11.69; N, 3.23. $C_{19}H_{43}O_3NSi_2$ requires C, 58.57; H, 11.13; N, 3.60); δ_H ($CDCl_3$) 0.05 (12H, s), 0.89 (18H, s), 1.32 (3H, s), 1.36 (3H, s), 1.39 (3H, s), 1.48 (3H, s), 2.85 (2H, broad), 3.75 (8H, s), 3.8-4.15 (4H, m).

2,2,4-Trimethyl-4-(tertiobutyldimethylsilyloxy)methyloxazolidin-3-oxyl (13) and 2-(tertiobutyldimethylsilyloxy)methyl-2,4,4-trimethyl-3-oxazolidinoxyl (14) were synthesized from **11** or **9** using a similar procedure as described for the direct oxidation of **8**. **13**, yield 18.2 %, ESR (EtOH) 3 lines, a_N = 14.8 G. ^{1}H NMR analysis of 2,2,4-trimethyl-4-(tertiobutyldimethylsilyloxy)methyl-oxazolidin-3-oxyl was realized after reduction of the N-O· group by phenylhydrazine. δ_H ($CDCl_3$) 0.05 (6H, s), 0.89 (9H, s), 1.25 (3H, s), 1.5 (6H, s), 2.1(1H, broad), 3.65 (4H, m). **14**, yield 24 %; ESR (EtOH): 3 lines, a_N = 14.8 G. ^{1}H NMR after reduction by phenylhydrazine: δ_H ($CDCl_3$) 0.05 (6H, s), 0.9 (9H, s), 1.15 (3H, s), 1.25 (3H, s), 1.35 (3H, s), 2 (1H, s), 3.4 (2H, s), 3.6-3.7 (2H, m).

2,4-dimethyl-2,4-(ditertiobutyldimethylsilyloxy)methyl-3-oxazolidinoxyl ($15_{a,b}$) has been obtained from $12_{a,b}$ using a similar procedure as described for **13** as a mixture of two diastereoisomers which were separated on a silica gel column with pentane-ether (90:10). The first diastereoisomer 15_a (R_f = 0.34) was obtained with 5.3 % yield: MS (E.I.) analysis: m/z 404 (M^+, 7 %); ESR (EtOH): 3 lines, a_N = 14.4 G. ^{1}H NMR ($CDCl_3$) after reduction by phenylhydrazine: δ_H 0.05 (6H, s), 0.9 (9H, s), 1.2 (3H, s), 1.37 (3H, s), 3.48 (4H, s), 3.6-3.8 (2H, m). The second diastereoisomer 15_b (R_f = 0.53) was obtained in 2.7 % yield: M.S. (EI) analysis: m/z 404 (M^+, 12 %), 388 (M^+ - 16, 14 %), 374 (M^+ - 30, 11 %); ESR (EtOH): 3 lines, a_N = 14.4 G.

2,2,4-Trimethyl-4-(hydroxymethyl)-3-oxazolidinoxyl (1). To a stirred solution of nitroxide **13** (200 mg, 0.73 mmol) in tetrahydrofuran (4.6 ml) at O °C (dry ice bath) was added dropwise 4 ml of a 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran. The reaction was stirred during 4 h at room temperature. The mixture was diluted with ethyl acetate, washed with saturated aqueous sodium chloride, dried with anhydrous sodium sulfate and concentrated under reduced pressure. The residue was chromatographied on a silica gel column with dichloromethane-methanol (95:5) as eluant to give 43 mg (37 %) of nitroxide **1** as an yellow oil (Found: C, 52.41; H, 8.84; N, 8.60. $C_7H_{14}O_3N$ requires C, 52.47; H, 8.81; N, 8.75); MS (FAB) analysis: m/z 162 (MH_2^+, 20 %), 161 (MH^+, 1.5 %), 145 (MH^+ - 16, 3.5 %), 130 (MH^+ - 31, 24.6 %); ESR (EtOH): 3 lines, a_N = 15.5 G. ^{1}H NMR ($CDCl_3$) after reduction by phenylhydrazine: δ_H 1.22 (3H, s), 1.35 (6H, s), 2.05 (1H, s), 3.4 (2H, s), 3.6-3.9 (2H, s).

2-(Hydroxymethyl)-2,4,4-trimethyl-3-oxazolidinoxyl (2) and 2,4-dimethyl-2,4-(dihydroxymethyl)-3-oxazolidinoxyl (3) were synthesized from **14** and 15_a, respectively, using a similar manner as described for **1**. **2**, yield 12 %; MS (FAB) analysis: m/z 162 (MH_2^+, 16 %), 161 (MH^+, 1 %), 145 (MH^+ - 16, 3 %); ESR (EtOH): 3 lines, a_N = 15.6 G. ^{1}H NMR ($CDCl_3$) after reduction by phenylhydrazine: δ_H 1.2 (3H, s), 1.3 (3H, s), 1.4 (3H, s), 3.48 (2H, s), 3.55-3.7 (2H, m). **3**, yield 3%, ESR (EtOH): 3 lines, a_N = 14.5 G.

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