EPR and ENDOR of Sodium Complexes of Spin Labeled Monoazacrown Ethers

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Z. Naturforsch. **42b**, 376–380 (1987); received September 15, 1986

Azacrown Ethers, Nitroxyl Radicals, Synthesis, EPR, ENDOR

Sodium complexes **3** and **5** of the spin labeled monoazacrown ethers 2,2,15,15-tetramethyl-1-aza-4,7,10,13-tetraoxacyclopentadecane-1-oxyl (**2**) and 1,4,7,10-tetraoxa-13-(2′,2′,6′,6′-tetramethylpiperidine-1′-oxyl-4′-yl)azacyclopentadecane (**4**) were synthesized and studied by EPR and ENDOR spectroscopies. The hyperfine splitting parameter and the g value of the complex **5,** containing the exocyclic nitroxyl moiety, were identical to the parent complexon **4,** *i.e.,* a_N = 15.6 G, g = 2.0060 ± 0.0001. In contrast, the data for the complex **3,** containing the endocyclic nitroxyl moiety were different. Thus, the spectrum of the complex **3** was a triplet of quartets with a_N = 15.7 G, a_{Na} = 2.47 G, g = 2.0062 ± 0.0001, whereas the spectrum of the parent complexon **2** was a triplet with a_N = 15.5 G, g = 2.0059 ± 0.0001.

The interaction between the sodium cation and the nitroxyl moiety in **3** was further confirmed by the sodium ENDOR transition at 7.27 MHz.

Introduction

The naturally occurring ionophores, such as, monensin, nigericin, nonactin, valinomycin and others [1, 2] are of practical and scientific interest, partly, because of their biological activity, and partly, because of their ability to transport cations across cell membranes [2]. Unfortunately, these ionophores possess complex structures [1], and, hence, neither themselves nor their analogs are amenable to easy synthetic procurement. The introduction of synthetic crown ether ionophores in 1967 [3, 4] had a profound effect on various aspects of synthetic methodology, and on some aspects of biochemical research since crown ethers can mimic the natural ionophores in complexing of cations and in permeation through membranes [5]. Furthermore, in contrast to the natural complexons, a variety of crown ethers can be comparatively easily synthesized [6] to accomodate different cations by varying the size of the cavity and by inclusion of hetero atoms of various polarity into the ring structures. Therefore, it is not surprising that over the past two decades an extensive literature has been generated on the synthetic methodologies [6] for the production of a wide variety of crown ethers and on the thermodynamic and kinetic data of their interactions with cations [5, 7].

Surprisingly, in spite of this voluminous literature, only a comparatively small number of studies were published [8–12] dealing with the electron paramagnetic resonance spectroscopy (EPR) of metal complexes of spin (nitroxyl) labeled crown ethers, although such, suitably designed, complexes could be of value in studying structural details of crown ethers, their permeation mechanism through cell membranes, the rate of exchange of the cations with environment, and in monitoring various cations in biological systems without the requirement of optically transparent samples. To date, in spite of substantial synthetic efforts, the design of suitable spin labeled crown complexons and their cation complexes, has met only with a partial success. Thus, although in a few cases [8, 10, 12] an interaction of potassium cation with the nitroxyl moiety was observed by EPR, no such interaction could be detected by EPR with the sodium cation [8, 12]. Recently, we reported the synthesis of several complexes of non-spin labeled monoazacrown ethers and their analyses by ^{23}Na NMR spectroscopy [13], and of several uncomplexed spin labeled monoazacrown ethers [14]. Now we would like to report the synthesis of the sodium complexes of two spin labeled

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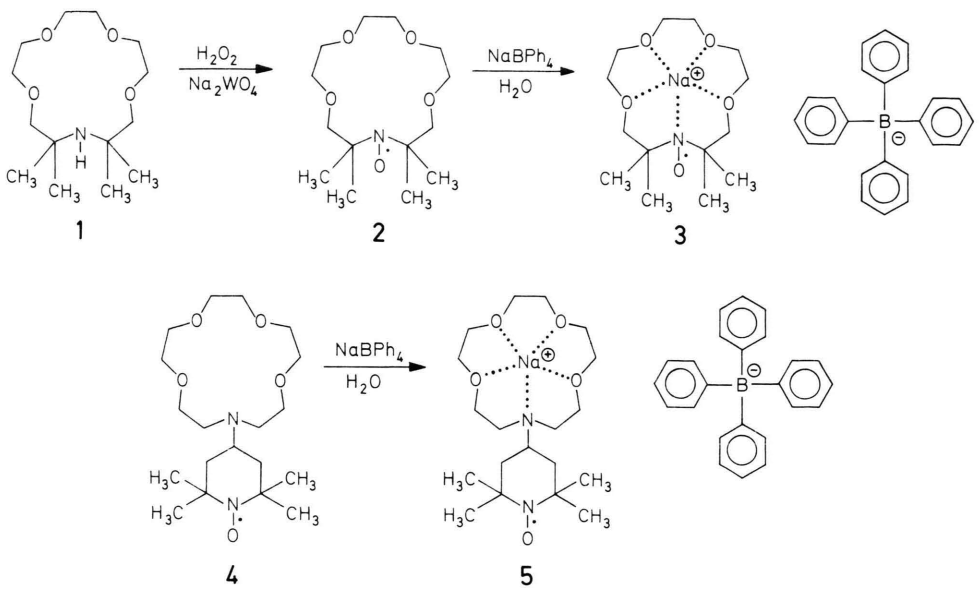
0340–5087/87/0300–0376/$ 01.00/0

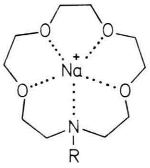


Scheme 1.

monoazacrown ethers, one (**3**) containing an endocyclic and the other (**5**) an exocyclic nitroxyl moiety, and the study of these complexes by EPR and ENDOR spectroscopies.

Results and Discussion

The EPR and ENDOR spectra of the sodium complex **3** must be recorded in aprotic solvents. The use of protic solvents, such as, ethanol causes an immediate exchange of the sodium cation, and as a result the spectrum of **3** consisting, in aprotic solvents, of a triplet of quartets is converted to a triplet of the parent compound **2.** This result may not be entirely surprising in view of the fact that sodium complexes of the monoazacrowns **6** and **7** possess low stabilities in methanol and aqueous methanol [7, 15–17]. Thus, the log K values for the complex **6** [7, 15] and **7** [7, 16, 17] were found to be 2.06 and 2.62, respectively. In complexon **2** the four methyl groups α to the nitrogen atom with their electron donating and steric properties, and the stabilized nitroxyl moiety which is a weaker Lewis base then the secondary and tertiary amino groups in **6** and **7** should have an effect on the $>\ddot{N}-\dot{O} \leftrightarrow >\overset{\cdot +}{N}-\overline{O}$ stability of the complex **3.** On the basis of space-filling models of **2** and **6,** the four methyl groups lock the complexon **2** into a fairly rigid ring structure with a smaller cavity than that in **6.** Hence, it is concluded that the stability of the complex **3** should be somewhat decreased as compared to that of **6** or **7.**



6: R = H
7: R = n-C_4H_9

The spectra of compounds **2** and **4** exhibited the expected interaction of the radical electron with the nitrogen nucleus as evidenced by the hyperfine splitting constants of about 15 Gauss (for exact values, see the experimental part). Similarly, the sodium complex **5,** in analogy to other investigations of spin labeled complexes of crown ethers [8, 12] exhibited no interaction of the sodium cation with the radical moiety. In contrast, there was observed an additional hyperfine splitting of the N-triplet of **3** into three quartets with an intensity of 1:1:1:1 (Fig. 1). It was assumed that the hyperfine splitting was caused by the sodium nucleus ($I = 3/2$) with a coupling constant $a_{Na} = 2.47\ G \triangleq 6.89\ MHz$ (Fig. 2).

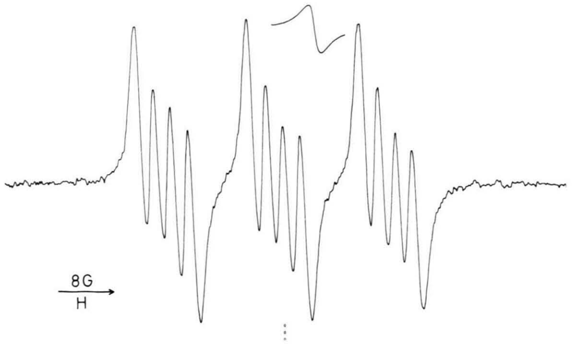


Fig. 1. The EPR spectrum of compound **3** in methylene chloride at room temperature. The EPR conditions were: 10 mW incident microwave power, 0.63 G modulation amplitude and 20 G/min scan rate. Strong pitch at g = 2.0028 was used as a reference.

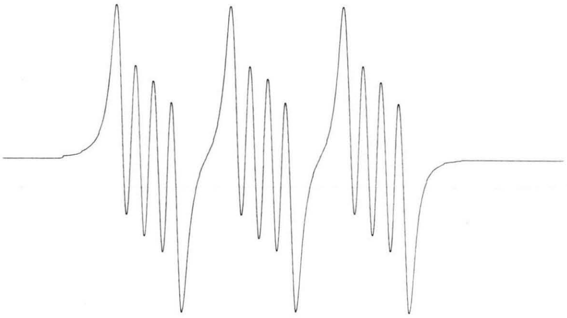


Fig. 2. Computer simulation of the hyperfine structure of compound **3** using a gaussian line shape.

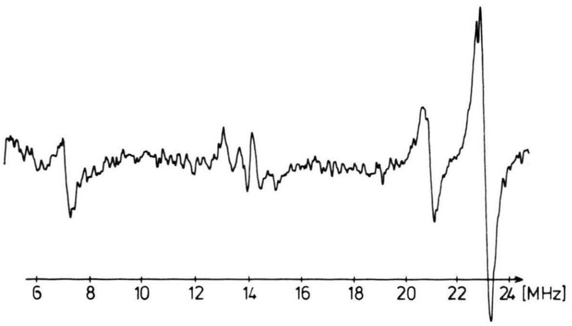


Fig. 3. The ENDOR spectrum of compound **3** in methylene chloride at 289 K.

In order to confirm directly the hyperfine structure of **3,** the ENDOR spectrum of **3** was recorded at a field strength of about 3280 G (Fig. 3). In this experiment the frequencies of the free nuclei of interest were located at $\nu_H = 13.95$ MHz, $\nu_N = 1.008$ MHz and $\nu_{Na} = 3.69$ MHz. Besides the proton resonances of the methyl groups located around ν_H, the transitions of the nitrogen nucleus at (1.008 ± 22.008) MHz = $(-)21.0$ MHz and 23.016 MHz were anticipated. The expectations were realized by the ENDOR values of 21.1 MHz and 23.2 MHz, whereby the small deviations from the calculated values are attributed to the temperature dependence of the nitrogen coupling. Analogous considerations for the postulated sodium frequencies resulted in values of (3.69 ± 3.44) MHz = 0.25 MHz and 7.13 MHz. While the lower frequency was not detected because of instrumental limitations, the higher transition at the frequency of 7.27 MHz was clearly visible. Thus, for the first time, the participation of the sodium cation in the molecular structure of a spin labeled monoazacrown complex (**3**) was established using EPR and ENDOR spectroscopies.

Experimental

Materials

All reagents were of the finest quality available commercially. Sodium tetraphenyl boron was purchased from MCB Reagents. The synthesis of the azacrown ethers **2** and **4** was briefly described earlier [14]. Now a detailed procedure is included.

Analytical procedures

Melting points were determined on a Thomas Hoover apparatus, model 6406-K with a calibrated thermometer. TLC analyses were performed on alumina oxide 60 F_{254} precoated sheets (type E), layer thickness 0.2 mm, with visualization using UV light and/or iodine chamber. For column chromatography basic alumina oxide, Brockmann activity 1, was used. Microanalyses were performed on a Perkin-Elmer Elemental Analyzer, model 240 C. Combustion difficulties have been experienced with the sodium complexes **3** and **5.** The IR spectra were recorded on a Nicolet 10-MX FTIR spectrophotometer. EPR spectra were obtained on a Varian E-115 ESR spectrometer, and a Varian E-109 Century Series X-band (9.3 GHz) spectrometer (Varian Instrument Division, Palo Alto, CA) with a dual rectangular (TE_{104}) cavity. A strong pitch standard (g = 2.0028) was placed in the reference cavity and was used to determine g values. The magnetic field was calibrated relative to an alkaline solution of potassium peroxylamine sulfonate (Fremy salt) at g = 2.0055 and a_N = 13.0 G. A Nicolet 1180 data system (Nicolet Instrument, Madison, WI) was used to stimulate spectra using the program ESRSIM. The ENDOR experiments were performed on a Varian E-Line spectrometer equipped with a Bruker Unit ER 810 and the Bruker data system ER 140.

Preparation of 2,2,15,15-tetramethyl-1-aza-4,7,10,13-tetraoxacyclopentadecane-1-oxyl (**2**)

Sodium metal (0.71 g, 31.0 mmol) was added to a stirred solution of N,N-bis(2-methyl-3-hydroxypropyl-2)amine [18] (1.61 g, 10.0 mmol) in *t*-butanol (75 ml), and the reaction mixture was heated with reflux until a clear solution resulted. After cooling the reaction mixture to 40 °C a solution of triethylene glycol bis-toluenesulfonate [19] (4.58 g, 10.0 mmol) in dry tetrahydrofuran (50 ml) was added dropwise over a period of 10 min, followed by 40 h of stirring at 40 °C. The solid precipitate was removed by filtration, and washed with ethyl ether (2×25 ml) and the combined filtrates and washings were concentrated on a rotating evaporator at 30 °C/20 torr. The yellow, semisolid residue was dissolved in water (40 ml), extracted once with *n*-hexane (30 ml) and the hexane extract discarded. The aqueous solution was extracted with dichloromethane (4×25 ml). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated at 30 °C/20 torr. The distillation of the residue in a Kugelrohr apparatus afforded 2.10 g, of a crude azacrown ether **1**, b.p. 108–118 °C/0.05 torr. Repeated Kugelrohr distillations of this material gave 1.30 g (47%) of pure **1**, b.p. 102–106 °C/0.015 torr, MS: *m/e* = 276 (M^++1, 100%). Purity control by TLC analyses (alumina; methanol) of this product resulted in a single spot. Without further analyses this intermediate (**1**) was oxidized to the spin labeled azacrown ether **2**. Thus, to the solution of **1** (0.308 g, 1.12 mmol) in distilled water (5 ml), containing dissolved sodium tungstate (30.0 mg), was added a 30% aqueous hydrogen peroxide (1.20 ml), and the resultant solution was kept for 40 h at 25 °C. The yellow solution was extracted with chloroform (3×2 ml), the combined organic extracts dried with anhydrous magnesium sulfate and concentrated at 40 °C/20 torr. Column chromatography of the crude product **2** on basic alumina (Brockmann, activity 1) using *t*-butyl methyl ether as eluant afforded 0.101 g (31%) of pure product **2**, m.p. 46–47 °C (crystallization: *n*-hexane, −25 °C).

MS: *m/e* = 291 (M^++1, 100%), 290 (M^+, 90%); IR (KBr): ν_{max} = 1115, 1141, 1356, 2900–2980 cm^{-1}; EPR (c = 0.41 mM; CH_2Cl_2, or C_2H_5OH, or 0.1 mM; PhMe): 3 lines, a_N = 15.5 G; g = 2.0059 ± 0.0001.

$C_{14}H_{28}NO_5$ (290.38)

Calcd	C 57.90	H 9.72	N 4.82,
Found	C 57.82	H 9.94	N 4.66.

Preparation of 1,4,7,10-tetraoxa-13-(2′,2′,6′,6′-tetramethylpiperidine-1′-oxyl-4′-yl)azacyclopentadecane (**4**)

A solution of 4[bis(2-hydroxyethyl)]amino-2,2,6,6-tetramethylpiperidine-1-oxyl [20, 21] (0.65 g, 2.5 mmol) and triethylene glycol bis-*p*-toluenesulfonate [19] (1.15 g, 2.5 mmol) in dry dimethylformamide (10 ml) was added dropwise during a period of 10 min to a suspension of sodium hydride [a 50% suspension in mineral oil, 0.24 g, 5.0 mmol, this material was washed with *n*-hexane (3×15 ml) prior to the use in the reaction] in dry dimethylformamide (10 ml). Following the addition, the reaction mixture was stirred for 24 h at 25 °C and then concentrated a rotating evaporator at 60 °C/20 torr. The oily residue was dissolved in water (20 ml) and extracted with chloroform (3×20 ml). The combined extracts were dried with magnesium sulfate and filtered. Concentration of the filtrate on a rotating evaporator at 30 °C/20 torr gave a crude product **4**. This material was further purified by column chromatography on alumina using a mixture of *t*-butyl methyl ether and acetone (17:3, v/v) as eluant. The red, oily product **4**, 0.43 g (45%) was dried for 20 h at 25 °C/0.03 torr.

MS: *m/e* = 374 (M^++1, 100%); IR (film): ν_{max} = 1124, 1359, 2863, 3500–3600 cm^{-1}; EPR (c = 0.3 mM; CH_2Cl_2, or C_2H_5OH, or 0.1 mM; PhMe): 3 lines, a_N = 15.6 G; g = 2.0060 ± 0.0001.

$C_{19}H_{37}N_2O_5 \times \frac{1}{2} H_2O$ (382.52)

Calcd	C 59.65	H 10.01	N 7.32,
Found	C 59.89	H 10.10	N 7.12.

Preparation of the sodium complexes **3** *and* **5** *of the spin labeled monoazacrown ethers* **2** *and* **4**, *respectively. A general procedure*

To a stirred solution of sodium tetraphenyl boron (10.0 mg, 29 μM) in water (0.40 ml) was added rapidly a solution of either 2,2,15,15-tetramethyl-1-aza-4,7,10,13-tetraoxacyclopentadecane-1-oxyl (**2**, 7.3 mg, 25 μM) or 1,4,7,10-tetraoxa-13-(2′,2′,6′,6′-tetramethylpiperidine-1′-oxyl-4′-yl)azacyclopentadecane (**4**, 9.5 mg, 25 μM) in water (0.25 ml). The solutions became instantly turbid. They were stirred at 25 °C until crystalline precipitates were formed

(1–4 h). The products were collected by filtration, washed with water (3×0.50 ml), dry ethyl ether (2×1.0 ml), and dried at 25 °C/0.1 torr.

The yields and analytical date were as follows:

3: 60%, m.p. 192–194 °C (dec.); IR (KBr): ν_{max} = 1074, 1095, 1120, 1359, 1384, 1429, 2932, 3054 cm^{-1}; EPR (c = 0.03 mM; CH_2Cl_2): a triplet of quartets; g = 2.00062 ± 0.0001; a_N = 15.7 G, a_{Na} = 2.47 G; (c = 0.03 mM, C_2H_5OH): a triplet; a_{Na} = 15.7 G.

$C_{38}H_{48}BNaNO_5$ (632.62)
Calcd C 72.14 H 7.65 N 2.21,
Found C 72.14 H 8.19 N 2.13.

5: 38%, m.p. 188–193 °C (dec.), sinters at 165 °C; IR (KBr): ν_{max} = 1066, 1106, 1118, 1245, 1354, 1384, 1428, 1459, 1478, 1580 cm^{-1}; EPR (c = 0.3 mM, CH_2Cl_2 or C_2H_5OH): 3 lines, a_N = 15.6 G; g = 2.0060 ± 0.0001.

$C_{43}H_{57}BNaN_2O_5$ (715.75)
Calcd C 72.15 H 8.03 N 3.91,
Found C 71.11 H 8.34 N 3.76.

The author, G. Sosnovsky, thanks the Graduate School of the University of Wisconsin-Milwaukee for the J. D. and D. Shaw Awards in 1983, 1984, 1985 and 1986 for NMR imaging.

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Addendum in press

Recently, it was shown, M. Delgado, L. Echegoyen, V. J. Gatto, D. A. Gustowski, and G. W. Gokel, J. Am. Chem. Soc. **108,** 4135 (1986) that the electrolytic reduction of the nitro group in *N*-(2-nitrobenzyl)aza-15-crown produces a radical anion which couples with Li^+, Na^+, and K^+ of the crown complex. Interestingly, the ESR spectrum exhibits a very large metal atom splitting A_{Na} = 2.46 G which is identical with our value involving the coupling of crown sodium with the endocyclic nitroxyl moiety.

Nachdruck – auch auszugsweise – nur mit schriftlicher Genehmigung des Verlages gestattet
Satz und Druck: Allgäuer Zeitungsverlag GmbH, Kempten