Synthesis and crystal structure of dinitroxyl crown ether biradical with indole groups in the side chains

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Dinitroxyl crown ether biradical, 4,13-bis[2-hydroxy-3-(2,2,4,4-tetramethyl-3-oxylo-1,2,3,4-tetrahydro-γ-carbol-9-yl)propyl]-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane, was synthesized and studied by X-ray structural analysis and ESR spectroscopy. The ESR spectra in aqueous and aqueous-alcoholic solutions show a triplet with a_N = 1.65±0.05 mT. This triplet is retained in the presence of Na^+, K^+, and Mg^{2+} ions. On addition of Ca^{2+} ions, the triplet is transformed into the quintet because of the change in the conformation of the crown ether biradical and decrease in the distance between the nitroxyl groups. The new crown ether biradical containing hydrophobic indole groups may be used for studying the mechanism of Ca^{2+} ion transport in biomembranes by ESR spectroscopy.

Key words: dinitroxyl crown ether biradical, X-ray structural study, ESR spectroscopy, Ca^{2+} ion transport.

Previously,[1,2] the synthesis of dinitroxyl crown ether biradicals was reported. Unlike crown ether monoradicals,[3–6] the ESR spectra of crown ether biradicals are more informative because the triplet ESR spectrum transforms into the quintet spectrum upon complexation with Ca^{2+} and Cd^{2+} ions.[1] This occurs because of the spatial approach of the nitroxyl groups, which is caused by flattening of the crown ether molecule and, apparently, by an additional coordination interaction between ions of bivalent metals and hydroxyl groups of the side chains of the crown ether biradical. However, dinitroxyl derivatives of crown ethers, which we have synthesized previously,[1] are hydrophilic and are distributed in the intermembrane space of cells and cell organelles (mitochondria and chloroplasts). Both hydrophilic and partially hydrophobic dinitroxyl derivatives of crown ethers, which possess membranotropic properties, are necessary for biophysical studies of Ca^{2+} ions. The aim of this work was to synthesize the crown ether biradical containing hydrophobic paramagnetic fragments and which can be incorporated into the biological membrane and can change its conformation and the ESR spectra selectively under the effect of Ca^{2+} ions in the presence of Na^+, K^+, and Mg^{2+} ions.

We have synthesized crown ether **4** modified with nitroxyl radicals of partially hydrogenated γ-carboline[7,8] which have previously been used as spin probes that interact with hydrophobic regions of albumin[9] (Scheme 1).

It was established by X-ray structural study that in the crystals the molecule of crown ether **4** is centrosymmetric, *i.e.*, this molecule is a *meso* isomer [the C(21) and C(21′) chiral centers have opposite configurations] (Fig. 1). It was found that in the crystal the H atom and the OH group at the C(21) atom are disordered between two positions with occupancies of 0.7 and 0.3 (see Fig. 1). It is evident that interchange of the positions of the H and OH atoms reverses the chirality of the C(21) atom.

The conformation of the 18-membered heterocycle is $g^+tg^+g^-ttg^+g^-tg^-tg^-g^+ttg^-g^+t$ (Table 1). Apparently, the formation of intramolecular hydrogen bonds between the heteroatoms of the macrocycle and the hydroxyl groups of the side chains (see Fig. 1, disordered atoms with occupancies of 30 % are primed) is favorable for this conformation. In the molecule with the higher occupancy, the bifurcated hydrogen bond is formed, O(21)—H...N(11) [O...N 2.851(3), H...N 2.57(3) Å, O—H...N angle 99(1)°] and O(21)—H...O(17a) [O...O 2.931(3), H...O 2.05(3) Å, O—H...O angle 175(1)°], which closes the five-membered cycle, whereas in the molecule with the lower occupancy, the O(21′)—H...N(11) hydrogen bond [O...N 2.897(6), H...N 2.44(6) Å, O—H...N angle 107(2)°] is formed, which also closes the five-membered cycle. All heteroatoms of

† Deceased.

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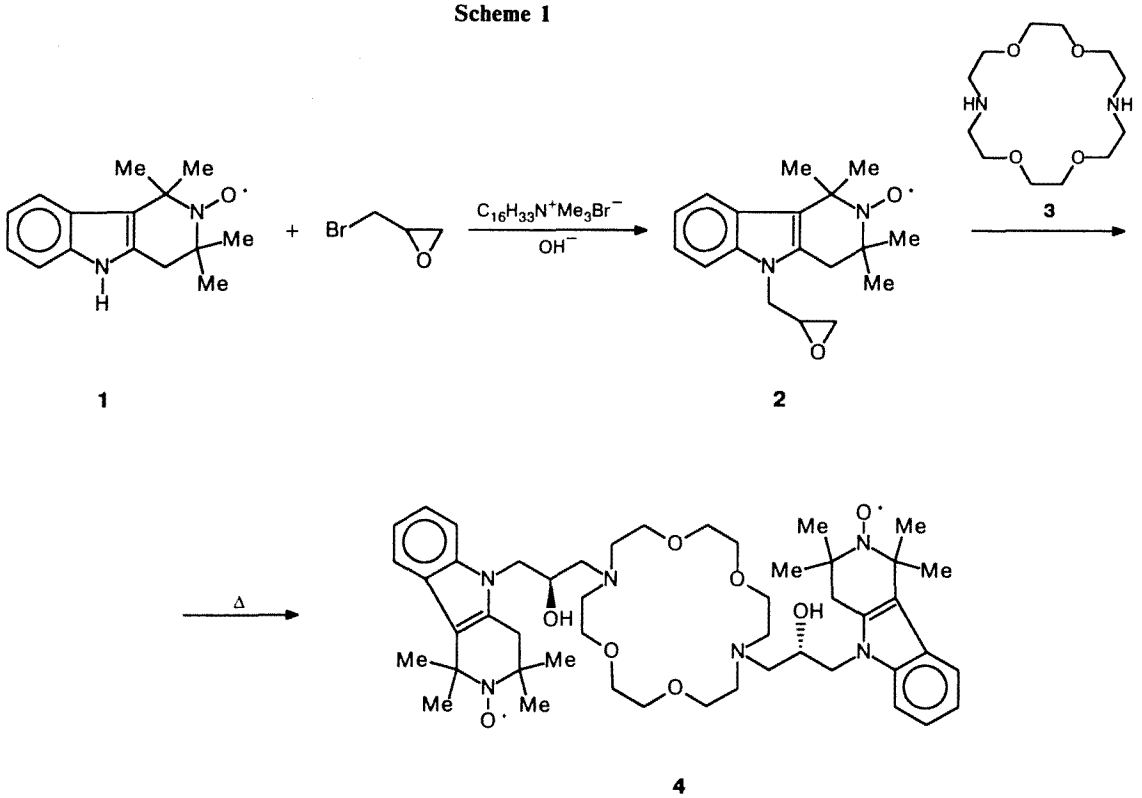
Scheme 1



the crown ether cycle are in a single plane (the rms deviation of the atoms from the mean plane is 0.09 Å); this plane is virtually parallel to the mean planes of *N*-carboline substituents in the side chains (the dihedral angle is 7.2°); the N(11) atom of the crown-ether cycle has a pyramidal bond configuration (the sum of the bond angles is 338.8°). The tetrahydro-γ-carboline fragment is virtually planar (the rms deviation of the atoms from the mean plane is 0.04 Å) except for the saturated C(3) atom, which deviates from the plane of the remaining atoms of the tetrahydrocarboline fragment by 0.49 Å. The N(1) and N(4) atoms have a planar-trigonal bond configuration (the sums of the bond angles are 359.9° and 356.0°, respectively).

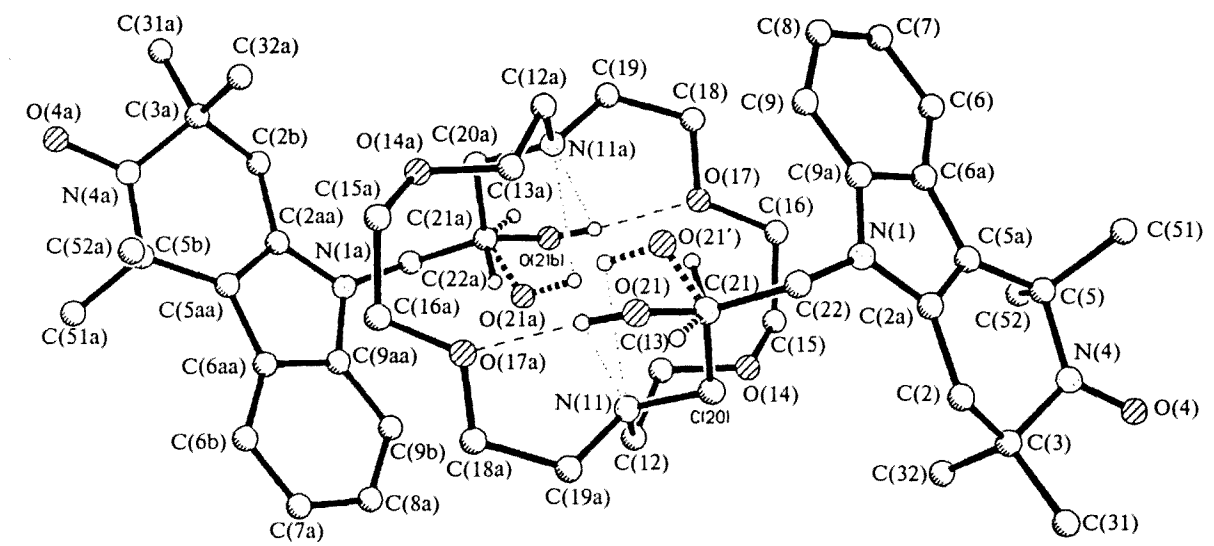


Fig. 1. Overall view of molecule **4**. Alternative positions of the H(21) atom and the H and O atoms of the hydroxyl groups with occupancies of 0.3 are primed; hydrogen bonds are indicated by dashed lines.

Table 1. Torsion angles (θ) in the crown cycle of molecule **4**

Angle	θ/deg
N(11)—C(12)—C(13)—O(14)	86.8(0.3)
C(12)—C(13)—O(14)—C(15)	168.5(0.2)
C(13)—O(14)—C(15)—C(16)	80.6(0.3)
C(14)—C(15)—C(16)—O(17)	−80.0(0.3)
C(15)—C(16)—O(17)—C(18)	164.4(0.2)
C(16)—O(17)—C(18)—C(19)	−173.0(0.2)
O(17)—C(18)—C(19)—N(11a)	63.0(0.3)
C(18)—C(19)—N(11a)—C(12a)	−87.4(0.2)
C(19)—N(11a)—C(12a)—C(13a)	159.2(0.2)

The intramolecular distance between the paramagnetic centers (O(4)...O(4a)) is 17.726(6) Å. The geometric parameters of molecule **4** have normal values[10] and are given in Tables 1—3.

The distances between the hydroxyl groups of the side chains and the center of the crown ether are 3.75 Å, which does not contradict the hypothesis that the coordination interaction of the Ca^{2+} ion with these groups is possible.

The ESR spectra of compound **4** in aqueous and aqueous-alcoholic solutions show triplets with a_N = 1.65±0.05 mT, g = 2.003±0.001. On addition of Ca^{2+} ions to a solution of **4**, the triplet transforms to the quintet. We did not find substantial differences in the ESR spectra of the complexes of Ca^{2+} with **4** and with the crown ether biradicals described previously.[1] As in the case of dinitroxyl derivatives of crown ethers,[1] the transformation of the triplet in **4** into the quintet under the action of Ca^{2+} ions occurs because of the change in the conformation of dinitroxyl derivatives of crown ethers and the decrease in the distance between the nitroxyl fragments. The triplet of the ESR spectrum of **4** is not transformed into the quintet in the presence of Na^+, K^+, and Mg^{2+} ions. The selectivity of crown ether biradicals toward the Ca^{2+} ions in the presence of Na^+, K^+, and Mg^{2+} ions makes it possible to use these biradicals for ESR studies of Ca^{2+} ion transport in membranes of chloroplasts, in which, in particular, light-dependent absorption and release of Ca^{2+} ions was observed.[11]

Table 2. Bond lengths (*d*) in molecule **4**

Bond	*d*/Å	Bond	*d*/Å	Bond	*d*/Å	Bond	*d*/Å
C(12a)—N(1)	1.386(3)	C(5)—C(51)	1.530(4)	C(9)—C(9a)	1.397(3)	C(16)—O(17)	1.423(4)
C(2a)—C(2)	1.494(3)	C(5)—C(52)	1.535(4)	C(9a)—N(1)	1.384(3)	O(17)—C(18)	1.422(3)
C(2a)—C(5a)	1.355(3)	C(5)—C(5a)	1.508(3)	N(11)—C(12)	1.462(4)	C(18)—C(19)	1.508(4)
C(2)—C(3)	1.535(4)	C(5a)—C(6a)	1.440(3)	N(11)—C(20)	1.463(3)	C(19)—N(11a)	1.470(4)
C(3)—C(31)	1.519(5)	C(6a)—C(6)	1.403(3)	N(11)—C(19a)	1.470(4)	C(20)—C(21)	1.506(3)
C(3)—C(32)	1.535(4)	C(6a)—C(9a)	1.410(3)	C(12)—C(13)	1.505(4)	C(21)—O(21)	1.383(4)
C(3)—N(4)	1.492(3)	C(6)—C(7)	1.378(4)	C(13)—O(14)	1.426(4)	C(21)—C(21′)	1.185(7)
N(4)—O(4)	1.288(3)	C(7)—C(8)	1.396(4)	O(14)—C(15)	1.426(4)	C(21)—C(22)	1.528(4)
N(4)—C(5)	1.498(3)	C(8)—C(9)	1.380(4)	C(15)—C(16)	1.492(4)	C(22)—N(1)	1.460(3)

Table 3. Bond angles (ω) in molecule **4**

Angle	ω/deg	Angle	ω/deg	Angle	ω/deg
C(2a)—N(1)—C(9a)	107.7(2)	C(51)—C(5)—C(52)	109.3(2)	C(6)—C(6a)—C(9a)	118.8(2)
C(2a)—N(1)—C(22)	127.6(2)	N(4)—C(5)—C(5a)	108.2(2)	C(20)—N(11)—C(19a)	113.8(2)
C(9a)—N(1)—C(22)	124.6(2)	C(51)—C(5)—C(5a)	110.5(2)	N(11)—C(12)—C(13)	114.7(2)
N(1)—C(2a)—C(2)	124.8(2)	C(52)—C(5)—C(5a)	112.1(2)	C(12)—C(13)—O(14)	110.7(2)
N(1)—C(2a)—C(5a)	110.2(2)	C(2a)—C(5a)—C(5)	124.4(2)	C(13)—O(14)—C(15)	113.8(2)
C(2)—C(2a)—C(5a)	124.9(2)	C(2a)—C(5a)—C(6a)	107.5(2)	O(14)—C(15)—C(16)	114.2(2)
C(2a)—C(2)—C(3)	110.3(2)	C(5)—C(5a)—C(6a)	127.9(2)	C(15)—C(16)—O(17)	110.6(2)
C(2)—C(3)—C(31)	109.8(2)	C(5a)—C(6a)—C(6)	135.3(2)	C(16)—O(17)—C(18)	111.9(2)
C(2)—C(3)—C(32)	110.6(2)	C(5a)—C(6a)—C(9a)	106.0(2)	O(17)—C(18)—C(19)	108.3(2)
C(31)—C(3)—C(32)	110.1(2)	C(6a)—C(6)—C(7)	118.9(2)	C(18)—C(19)—N(11a)	111.6(2)
C(2)—C(3)—N(4)	109.4(2)	C(6)—C(7)—C(8)	121.1(2)	N(11)—C(20)—C(21)	110.7(2)
C(31)—C(3)—N(4)	108.0(2)	C(7)—C(8)—C(9)	122.0(2)	C(20)—C(21)—O(21)	115.3(2)
C(32)—C(3)—N(4)	108.9(2)	C(8)—C(9)—C(9a)	116.6(2)	C(20)—C(21)—O(21′)	130.0(4)
C(3)—N(4)—O(4)	116.8(2)	N(1)—C(9a)—C(6a)	108.5(2)	C(20)—C(21)—C(22)	113.3(2)
C(3)—N(4)—C(5)	124.1(2)	N(1)—C(9a)—C(9)	128.8(2)	O(21)—C(21)—C(22)	112.1(3)
O(4)—N(4)—C(5)	115.1(2)	C(6a)—C(9a)—C(9)	122.6(2)	O(21′)—C(21)—C(22)	108.4(3)
N(4)—C(5)—C(51)	106.9(2)	C(12)—N(11)—C(20)	114.4(2)	N(1)—C(22)—C(21)	112.9(2)
N(4)—C(5)—C(52)	109.6(2)	C(12)—N(11)—C(19a)	110.6(2)		

Table 4. Atomic coordinates of nonhydrogen atoms ($\times 10^4$) in molecule **4**

Atom	*x*	*y*	*z*	Atom	*x*	*y*	*z*	Atom	*x*	*y*	*z*
N(1)	972(2)	3273(2)	3649(1)	C(5a)	2816(2)	2791(2)	3140(1)	C(15)	3701(3)	−486(3)	4337(1)
C(2a)	2359(2)	3540(2)	3565(1)	C(6a)	1664(2)	1997(2)	2930(1)	C(16)	2608(3)	−850(3)	3857(1)
C(2)	3201(3)	4560(3)	3874(1)	C(6)	1468(3)	1042(2)	2508(1)	O(17)	1610(2)	−1671(2)	4080(1)
C(3)	4776(3)	4302(3)	3859(1)	C(7)	164(3)	495(2)	2413(1)	C(18)	371(3)	−1773(3)	3687(1)
C(31)	5603(4)	5492(4)	3995(1)	C(8)	−957(3)	888(3)	2723(1)	C(19)	−691(3)	−2525(3)	3990(1)
C(32)	5257(3)	3285(4)	4308(1)	C(9)	−802(2)	1803(3)	3153(1)	C(20)	1310(3)	2751(3)	4984(1)
N(4)	5059(2)	3878(2)	3252(1)	C(9a)	530(2)	2337(2)	3255(1)	C(21)	−53(3)	2984(3)	4615(1)
O(4)	6302(2)	4077(2)	3095(1)	N(11)	1098(2)	1900(2)	5473(1)	O(21)	−1165(3)	3341(3)	4928(1)
C(5)	4252(2)	2870(2)	2916(1)	C(12)	2264(3)	1042(3)	5620(1)	O(21′)	−1021(6)	2312(6)	4498(3)
C(51)	4136(3)	3248(3)	2261(1)	C(13)	2430(3)	41(3)	5166(1)	C(22)	108(3)	3829(3)	4081(1)
C(52)	5058(3)	1635(3)	2992(1)	O(14)	3272(2)	475(2)	4718(1)				

Table 5. Atomic coordinates of hydrogen atoms ($\times 10^3$) in molecule **4**

Atom	*x*	*y*	*z*	Atom	*x*	*y*	*z*	Atom	*x*	*y*	*z*
H(21)	−32(4)	218(4)	445(2)	H(51b)	502(3)	338(2)	212(1)	H(15a)	406(3)	−122(2)	459(1)
H(21′)	−47	356	487	H(51c)	364(3)	255(2)	201(1)	H(15b)	455(3)	−14(2)	413(1)
H(21O)	−135(4)	284(4)	522(2)	H(52a)	515(2)	136(2)	342(1)	H(16a)	299(3)	−127(2)	354(1)
H(21′O)	−111	174	485	H(52b)	455(3)	96(2)	276(1)	H(16b)	215(3)	−7(2)	367(1)
H(2a)	299(3)	467(2)	429(1)	H(52c)	598(3)	174(2)	283(1)	H(18a)	59(3)	−216(2)	332(1)
H(2b)	293(3)	536(2)	367(1)	H(6a)	217(3)	76(2)	228(1)	H(18b)	1(2)	−86(2)	357(1)
H(31a)	543(3)	607(2)	372(1)	H(7a)	4(2)	−16(2)	213(1)	H(19a)	−25(3)	−325(2)	412(1)
H(31b)	532(3)	587(2)	439(1)	H(8a)	−182(3)	49(2)	264(1)	H(19b)	−158(3)	−275(2)	369(1)
H(31c)	666(3)	538(2)	397(1)	H(9a)	−157(2)	205(2)	338(1)	H(20a)	199(3)	242(2)	472(1)
H(32a)	461(3)	257(2)	428(1)	H(12a)	207(3)	59(2)	599(1)	H(20b)	172(3)	357(2)	514(1)
H(32b)	621(3)	308(2)	426(1)	H(12b)	317(3)	150(2)	567(1)	H(22a)	−80(3)	406(2)	389(1)
H(32c)	525(3)	360(2)	471(1)	H(13a)	294(3)	−72(2)	536(1)	H(22b)	55(3)	460(3)	422(1)
H(51a)	362(3)	404(2)	219(1)	H(13b)	153(3)	−22(2)	498(1)				

Experimental

The molecular weights of the compounds were determined on a LKB-9000 (Sweden) mass spectrometer. The ESR spectra were recorded on a Varian E-104 instrument in aqueous and aqueous-alcoholic solutions at concentrations of $0.5 \cdot 10^{-4}$—$1 \cdot 10^{-4}$ mol L^{-1}.

2,2,4,4-Tetramethyl-9-(2,3-epoxypropyl)-1,2,3,4-tetrahydro-γ-carboline-3-oxyl (2). A 50 % NaOH solution (8 mL), epibromohydrin (5 mL, 60 mmol), and cetyltrimethylammonium bromide (0.02 g) were added to a solution of compound **1** [7] (4.86 g, 20 mmol) in 25 mL of benzene. The reaction mixture was stirred at 60—70 °C for 5 h. After cooling, water was added (50 mL), and extraction with benzene was performed. Benzene was removed *in vacuo*, and the residue was chromatographed on a column with silica gel (L 40/100 μm) using $CHCl_3$ as an eluent, and a rose-colored fraction was collected. The solvent was removed *in vacuo*. Compound **2** was obtained as orange crystals (the yield was 3.54 g, 59 %), m.p. 127—128 °C (from EtOH). Found (%): C, 72.29; H, 7.77; N, 9.41. Mol. weight 299 (mass spectrometry). $C_{18}H_{23}N_2O_2$. Calculated (%): C, 72.21; H, 7.74; N, 9.36. Mol. weight 299.

Crown ether biradical 4. A mixture of epoxide **2** (0.6 g, 2 mmol) and diaza-18-crown-6 **3** (0.26 g, 1 mmol) was dissolved in 12 mL of EtOH. The solution was kept at 65 °C for 120 h. Ethanol was removed *in vacuo*, and the residue was chromatographed on a column with Al_2O_3 (elution with $CHCl_3$). Compound **4** was obtained as orange crystals (the yield was 0.48 g, 57 %), m.p. 162 °C (from EtOH). Found (%): C, 66.78; H, 8.47; N, 9.69. Mol. weight 860 (mass spectrometry). $C_{48}H_{72}N_6O_8$. Calculated (%): C, 66.94; H, 8.43; N, 9.76. Mol. weight 861.

X-ray structural study of compound 4. Crystals of compound **4** are monoclinic, space group $P2_1/c$, at 20 °C: a = 9.559(4), b = 10.731(4), c = 22.628(8) Å, β = 95.01(1)°, V = 2312(1) Å^3, Z = 2 (the molecule is located in an inversion center), d_{calc} = 1.030 g cm^{-3}, μ = 0.08 mm^{-1}. $C_{48}H_{72}N_6O_8$. Mol. weight 861.12. The unit cell parameters and intensities of reflections were measured on an automated four-circle Siemens P3/PC diffractometer (T = 20 °C, Mo-Kα radiation, graphite monochromator, θ/2θ scanning technique, θ_{max} = 27°). The structure was solved by the direct method and refined by the full-matrix least-squares method with anisotropic thermal parameters for nonhydrogen atoms. Oxygen atoms of hydroxyl groups are disordered between two positions with occupancies of 0.7 and 0.3; the position with occupancy 0.3 was refined isotropically. Hydrogen atoms were located from difference Fourier synthesis and were included in the refinement with fixed thermal parameters (U_{iso} = 0.04 Å^2). In addition, the disordered H(21′) and H(21′O) atoms at the C(21) and O(21′) atoms, respectively, with occupancies of 0.3, were refined with the fixed position parameters using the riding model. The atomic coordinates are given in Tables 4 and 5. The final values of R factors are as follows: R = 0.050, R_w = 0.049 using 3300 independent reflections with $I > 3\sigma(I)$. All calculations were performed on an IBM PC/AT-286 computer using the SHELXTL PLUS program package.[12]

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