# 4',4"- AND 4',5"-DI-sec-BUTYL-2,3:11,12-DIBENZO-18-CROWN-6 — AN IONOPHORE FOR MAGNESIUM IN NATURAL AND MODEL SYSTEMS

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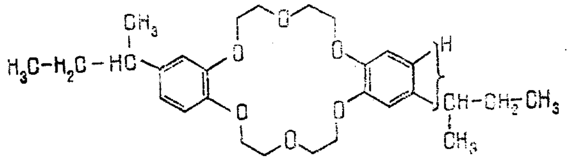
The ionophoric properties of the di-sec-butyl derivatives of dibenzo-18-crown-6 have been studied in natural and model membranes. It has been shown that the di-sec-butyl substituents in the benzene rings of dibenzo-18-crown-6 impart to the cyclopolyether the properties of an ionophore for magnesium. The $Mg^{2+}/Ca^{2+}$ selectivity of the cyclopolyether investigated was 4.8 according to results obtained on artifical membranes and mitochondria.

Natural and synthetic membrane-active compounds capable of selectively modifying the permeability of biological membranes have found wide use in various spheres of science and practice [1]. Among them, substantial interest is presented by the macrocyclic polyethers, which are a convenient model for elucidating the dependence of ionophoric properties on features of chemical structure. We have established previously [2-4] that diacyl derivatives of dibenzo-18-crown-6* exhibit the activity of calcium ionophores in biological membranes, while dialkyl- or di-α-hydroxyalkyl-substituted derivatives of this cyclopolyether induce permeability mainly for univalent cations.

We have established that introduction into the benzene rings of dibenzo-18-crown-6 of secondary butyl radicals imparts to the cyclopolyether the properties of an ionophore for magnesium ions. It is known that $Mg^{2+}$ plays an important role in the mechanism of the transport of ions through biological membranes in regulating the activity of many enzyme processes and the metabolism as a whole. In view of this, the discovery of an ionophore capable of modifying the permeability of membranes for this cation creates the possibility of directed modification of $Mg^{2+}$-dependent processes and activities.

In this investigation we used four preparation (I-IV) of di-sec-butyl derivatives of dibenzo-18-crown-6 consisting, according to NMR results, of mixtures of the structural isomers 4',4"- and 4',5"-di-sec-butyldibenzo-18-crown-6:



These preparations had mp 82-87°C, 78-84°C, 78-84°C, and 80-85°C, respectively. Compound (1) was obtained by the alkylation of dibenzo-18-crown-6 with n-butanol, and compounds (2-4) by its alkylation with secondary butanol in the presence of polyphophoric acid. In the first reaction, the n-butyl radical isomerized to a secondary butyl radical because of which the products obtained by both methods consisted of derivatives with secondary butyl substituents. The mass spectrometry of the derivatives investigated showed the presence in compound 2 of a trisubstituted derivative as impurity.

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*According to Pedersen's classification [5]. The full name is: 2,3:11,12-dibenzo-1,4,7,-10,13,16-hexaoxacyclooctadeca-2,11-diene.

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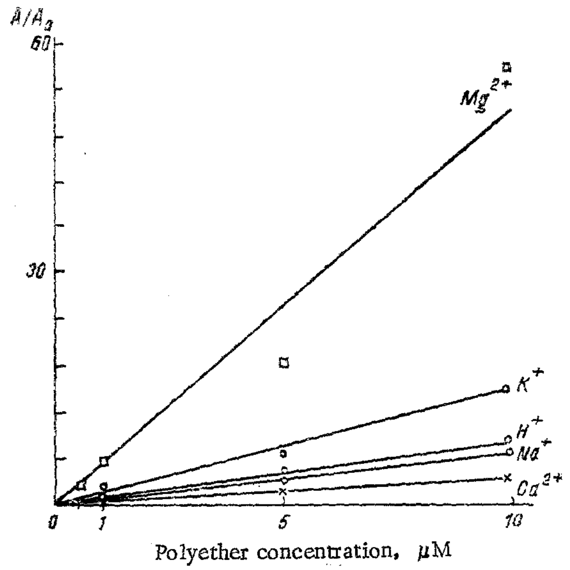

Fig. 1. Influence of various concentrations of compound (4) on the relative rate of transfer ($A/A_0$) of some uni- and bivalent cations through the inner membrane of rat liver mitochondria. The incubation medium with a volume of 3 ml contained isoosmotic concentrations of magnesium, calcium, potassium, and sodium nitrates (when the permeability for hydrogen ions was measured, an isoosmotic solution of ammonium nitrate was used), buffered with 10 mM Tris nitrate, pH 7.4, and 0.33 μg/ml each of antimin A and rotenone. Protein concentration — 1 mg/3 ml. A — rate of transfer in the presence of the ionophore; $A_0$ — in the control.

Figure 1 shows the effect of compound (4) on the permeability of inner mitochondrial membrane for $Mg^{2+}$, $Ca^{2+}$, and some univalent cations, measured from the rate of the energy-dependent swelling of the organelle in isoosmotic solution of the nitrate of these cations. The rate of swelling of the mitochondria in the different salt media increased proportionally to the concentration of the cyclopolyether (1-10 μM), the greatest increase in permeability being recorded for magnesium ion. In a concentration of 10 μM, compound (4) induced the permeability of the mitochondrial membranes in the sequence: $Mg^{2+}:K^{+}:H^{+}:Na^{+}:Ca^{2+} = 1:0.43:0.4:0.24:0.2$. Hence, the $Mg^{2+}/Ca^{2+}$ selectivity of this ionophore amounted to 4.8. We may note that natural ionophores of bivalent cations — lazalocid A, A 23187, and lysocillin — are characterized by a substantially smaller $Mg^{2+}/Ca^{2+}$ selectivity, which amounts to about 1 for A 23187 and lysocillin [6-9] and 0.13-0.38 for lazalocid A [10, 11]. Diacyl derivatives of dibenzo-18-crown-6, which are also ionophores for bivalent cations [2], have a $Mg^{2+}/Ca^{2+}$ selectivity of about 0.5, while the dibutyl derivative of this cyclopolyether or di-tert-butyl substituted dibenzo-30-crown-10 [12] scarcely affects the permeability of mitochondrial membranes for $Mg^{2+}$ ions.

In view of the existence of proton-transporting activity in di-sec-butyl derivatives of dibenzo-18-crown-6, they are uncoupling agents of oxidative phosphorylation in mitochondria, with a half-maximum effect at 10 μM.

As in the case of mitochondrial membranes, in the investigation of the action of compound (1) on the parameters of bilayer phospholipid membranes (BPMs), permeability for bivalent cations was detected. The cation specificity of BPMs modified by the cyclopolyether had the following sequence in this respect: $Mg^{2+}:Sr^{2+}:Mn^{2+}:Ca^{2+}:Ba^{2+} = 1:0.41:0.28:0.21:0.13$ (according to the magnitudes of the bionic potentials). Thus, as in the mitochondrial membranes, the $Mg^{2+}/Ca^{2+}$ selectivity of BPMs in the presence of the cyclopolyether is 4.8.

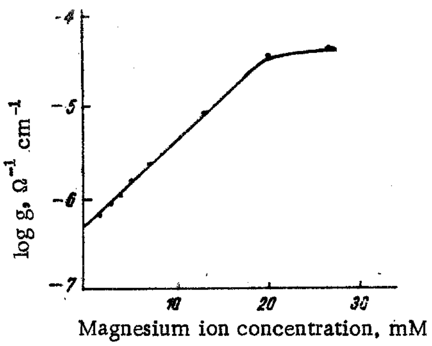

Fig. 2

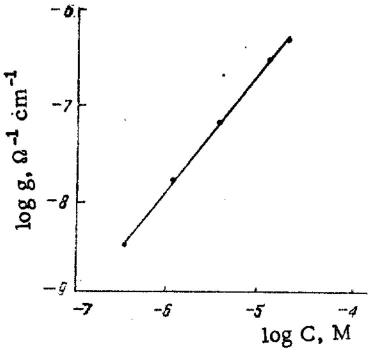

Fig. 3

Fig. 2. Dependence of the conductivity of BPMs on the concentration of magnesium ions in the incubation medium in the presence of compound (1). The incubation medium with a volume of 10 ml contained 25 mM tris-HCl, pH 7.0.

Fig. 3. Protonic conductivity of BPMs in the presence of compound (1). For the experimental conditions, see the caption to Fig. 2.

Figure 2 shows the dependence of the permeability of BPMs containing compound (1) on the concentration of $Mg^{2+}$ ions in the medium. The maximum value of the permeability of BPMs is established at 20 μM $MgCl_2$ in the medium, then being approximately two orders of magnitude higher than the control value. At the same time, compound (1) also caused a substantial increase in the permeability of the BPMs for $H^+$ (Fig. 3), which correlates with the effect of the cyclopolyether on the functions of the mitochondria.

Magnesium ions play an important role in the mechanism of the accumulation of $Ca^{2+}$ by the vesicles of the sarcoplasmic reticulum [13]. We have investigated the action of the di-sec-butyl derivatives of dibenzo-18-crown-6 on the accumulation of $Ca^{2+}$ in the sarcoplasmic reticulum of rabbit skeletal muscle.

In the processes of accumulation of $Ca^{2+}$, $Mg^{2+}$ ions apparently fulfill the role of counter-ions, compensating the transfer of $Ca^{2+}$ through the membrane, and the membrane of absorption of $Ca^{2+}$ in the sarcoplasmic reticulum is considered on the whole as a $Ca^{2+}/Mg^{2+}$ $(H^+)$-exchange system [14, 15]. The efficiency of the working of this system is evaluated directly from the $Ca^{2+}$/ATP ratio, which characterizes the amount of $Ca^{2+}$ ions that have accumulated in the hydrolysis of one molecule of ATP and is equal to 2 in the norm [13]. The coefficient $Ca^{2+}$/ATP falls with the aging of preparations of sarcoplasmic reticulum, under the action of ionophores capable of inducing $H^+/Ca^{2+}$ exchange, under the conditions of a deficiency of $Mg^{2+}$ in the medium, etc. [2, 13].

It follows from the results shown in Fig. 4 that compound (1) in a concentration of 50 μM increases the rate of transport of $Ca^{2+}$ severalfold, and its efficiency, evaluated from the $Ca^{2+}$/APT ratio, scarcely affects the $Ca^{2+}$-dependent ATPase activity of sarcoplasmic reticulum vesicles. The action of the cyclopolyether is most pronounced in the case of preparations of sarcoplasmic reticulum having a reduced $Ca^{2+}$/ATP ratio as the result of storage. It is obvious that in the process of storage of the sarcoplasmic reticulum the magnesium permeability of its membranes may be disturbed, and the use of a $Mg^{2+}$-ionophore restores it to a level ensuring the optimum activity of the calcium pump. The effect of the cyclopolyether described above was not imitated by classical protonophores and, consequently, it was due to an increase in the permeability of the membrane for magnesium ions.

Thus, the structural isomers of di(sec-butylbenzo)-18-crown-6 that we have investigated are effective membrane-active compounds capable of inducing permeability for $Mg^{2+}$ in both biological and model systems. The use of this ionophore on conjugated biomembranes generating an electrochemical potential of $H^+$ ions (mitochondria and chloroplasts) is partly limited by the existance in the cyclopolyether of a protonophoric activity, but this factor is unimportant in the case of other types of membranes (sacroplasmic reticulum, plasmatic membranes of eucaryotes, model systems), and the synthetic magnesium ionophore may be widely used for studying the mechanism of ion transport, regulation of metabolism, etc.

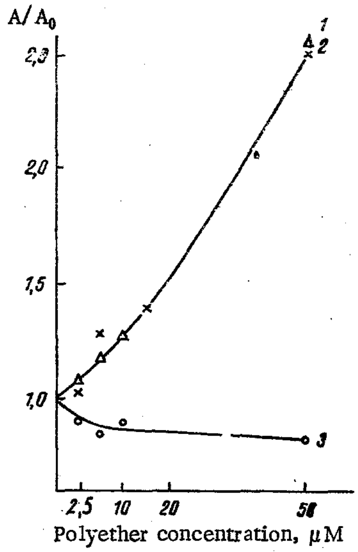

Fig. 4. Influence of various concentrations of compound (1) on the transport of $Ca^{2+}$ in the sarcoplasmic reticulum (along the axis of ordinates has been plotted the ratio of the parameters investigated in the experiment (A) and in the control ($A_0$)): 1) rate of transport of $Ca^{2+}$ (nmole of $Ca^{2+}$/mg of protein per min); 2) $Ca^{2+}$/ATP; 3) ATPase activity (nmole of $H^+$/mg of protein per min). In the control experiment, an equal volume of solvent (96% ethanol) was added in place of the cyclopolyether.

## EXPERIMENTAL

The isolation of mitochondria from rat liver, the determination of the permeability of the mitochondrial membranes from the rate of energy-independent swelling in isoosmotic solutions of nitrates of certain uni- and bivalent cations, the measurement of respiration in various metabolic states, and also the determination of the protein of the mitochondria and the sarcoplasmic reticulum have been described in a preceding paper [2]. The di-sec-butyl derivatives studied were dissolved in double-distilled ethanol. In all the experiments including bilayer phospholipid membranes an equal volume of double-distilled ethanol was added to the control samples in place of the compounds under investigation.

The isolation of the sarcoplasmic reticulum from the white muscles of the rabbit and the recording of the kinetics of the transport of $Ca^{2+}$ and $Ca^{2+}$-dependent ATPase activity by pH-metry were carried out as described previously [2].

The procedure for obtaining the artificial bimolecular membranes and determining their electrical parameters have been given elsewhere [3].

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