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Concerning the Relative Non-Toxicity of Silacrown Ionophores

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PANNELL, K. H., C. K. LA NEAVE, E. RICO AND B. ARKLES. *Concerning the relative non-toxicity of silacrown ionophores.* PHARMACOL BIOCHEM BEHAV **21**: Suppl. 1, 77–80, 1984.—Silacrowns, ionophoric materials in which an ethylene bridge of a normal crown ether has been replaced by a R_2Si group, facilitate ion transport across liquid membranes. A major aspect of their properties is their nontoxicity when compared to these crown ethers. This is probably due to their ready hydrolysis to the corresponding polyethylene glycols. The glycols also exhibit ion transport properties, even with an efficiency close to the silacrowns but the silacrowns stay intact long enough to partition into the hydrophobic phase of a liquid membrane whereas their hydrolysis products preferentially partition into the aqueous phases. Ready removal from the hydrophobic phase of a membrane will reduce the long term transporting effectiveness of the glycols. Thus, in vivo hydrolysis of the silacrowns will result in the loss of transmembrane carriers. Similar removal will not occur for normal crown ionophores which are both hydrolytically stable and partition into the hydrophobic phase, and remain there for long time periods.

Alkali metals Ionophores Ion transport Partitioning Polyethylene glycols Potassium Silacrown ethers Sodium

THERE has been a considerable recent interest in the use of synthetic ionophores with respect to elucidating the various factors that effect the efficiency and selectivity of ion transport across real and artificial membranes by ionophoric materials [3, 4, 5, 7, 10]. A major class of such synthetic compounds are the macrocyclic polyethers, the so-called crown ethers. A major drawback to the large scale use of such compounds, in both industrial and medicinal environments is their tendency to have harmful physiological properties. For example, several of the most useful crown ethers are toxic, as are various naturally occurring antibiotic ionophores, e.g., monensin. Presumably the ability of these species to slowly leak K^+ out of, and Na^+ into, the cell is responsible for this characteristic.

Recently a series of new ionophores based partially upon organosilicon chemistry have been reported [1]. In these compounds an ethylene bridge of a regular crown ether has been replaced by a disubstituted silyl group as illustrated in Fig. 1. Such substitution was expected to have several effects that may change the ion complexing and transporting ability of the resultant crown. For example, (a) size reduction of the central cavity, (b) change in the basicity of the crown due to the siloxy 0 atoms, and (c) reduction in the hydrolytic stability due to lability of siloxyethers.

We have reported that the efficiency of alkali metal ion binding and transporting of the silacrowns is reduced compared to the normal crown analogue and that the selectivity patterns are also changed. For example, whereas a normal crown with six oxygen atoms will usually be optimum for K^+, in the case of the silacrowns the seven oxygen atom ring is optimum [9]. Both of these properties seem to result from the presence of the two siloxy oxygen atoms. Such atoms are less basic than normal ethereal oxygens [2], thus the binding of the silacrowns to alkali metals will be less than the analogous non-substituted crown leading to less efficient transporting properties [7]. This feature will also create the need for a compensating O atom within the macrocycle ring as observed for optimum binding. Finally it has been demonstrated that the silacrowns are significantly less toxic than their unsubstituted analogues [1].

It is the purpose of this communication to report on the hydrolytic stability of the silacrown ionophores, and suggest a relationship between this and their physiological properties. The results indicate that judicious use of organosilicon chemistry may lead to the synthesis of very useful medicinal compounds.

METHOD

The silacrowns were synthesized at Petrarch Systems as previously described [1], and used in this laboratory without further treatment.

Rates of potassium picrate transport were obtained using the apparatus and procedures described elsewhere [7]. Concentrations of the ionophores in the hydrophobic phase and potassium picrate in the "in" aqueous phase were 10^{-2} m/l unless otherwise stated, and the temperature of the apparatus was maintained at 20°C. The "in" and "out" aqueous phases (10 ml) were buffered using TRISMA systems at pH=7, and the chloroform solvent for the hydrophobic phase (25 ml) was preequilibrated with buffered aqueous solutions for 24 hr prior to a transport run.

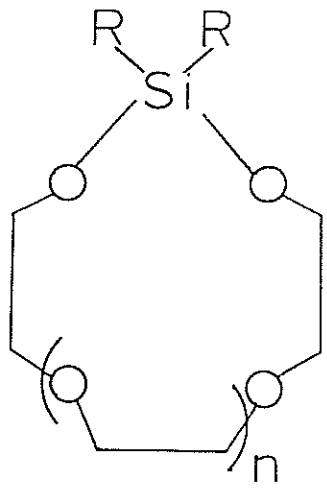

FIG. 1. Substitution of the ethylene bridge of a regular crown ether by a silyl group. Radical (R) represents phenyl, methyl, vinyl groups. n=1—Sila-11-Crown-4; 2—Sila-14-Crown-5; 3—Sila-17-Crown-6; 4—Sila-20-Crown-7.

TABLE 1

TRANSPORT OF POTASSIUM PICRATE, (M/l·MIN) 10^8

Carrier	Rate	Ratio Crown/Glycol
Sila-20-crown-7	400	5.7
Hexaethyleneglycol	70	
Sila-14-crown-5	19	9.5
Tetraethyleneglycol	2	

TABLE 2

HYDROLYSIS OF SILA-14-CROWN-5 IN ISOTONIC SALINE SOLUTION, pH 7.4*

Time (m)	% silacrown	% tetraethylene glycol†
0	100	—
5	90	—
25	44	34
66	37	38

*Analysis by liquid chromatographic analysis using a 500A styragel column, at 30°C.

†While tetraethylene glycol is the ultimate hydrolysis product, an intermediate product is readily detected, probably $Me_2Si(OH)(OCH_2CH_4)_4OH$. If the ratio of silacrown to isotonic saline solution is increased, e.g., to 5:1, then formation of the intermediate is the predominant initial reaction, with much less glycol formation. Conversely, if the ratio is reduced much less intermediate is found at any time, and glycol formation is dramatically enhanced.

In all experiments aliquots of the "out" aqueous phase were taken approximately every 20 m and the concentration of the picrate anion determined spectrophotometrically using the absorbance of the band at 357 nm. In all cases a plot of A versus t was linear over the time periods of our experiments, 80–200 m. From such slopes the rate of transport was determined. These rates are initial rates since we are transporting down a concentration gradient. The final concentration of the potassium salts in the "out" phase is in the range 10^{-4} m/l, thus no problems are encountered by a close approach to the equilibrium condition.

In experiments to determine the partitioning of the ionophores, both silacrowns and polyethylene glycols, a normal transport run was set up, *minus any salt in the "in" aqueous phase*. The set up was allowed to stir for periods of between 20 and 60 min. At this time the two aqueous phases were withdrawn and replaced with a salt solution in the "in" phase, and a buffered solution in the "out" phase and a normal K^+ transport run was commenced. If partitioning of the ionophore into the aqueous phase has taken place, the removal of these phases will cause a loss of ionophore, hence reduction in transport. Related experiments were performed with tetraethylene glycol and the unsubstituted crown ether, 15-C-5.

Studies on the hydrolysis of the silacrowns were performed in the following manner. An equal amount by volume of sila-14-C-5 and an isotonic saline solution (pH=7.4, 0.1% NaCl, 0.03% gelatin) were permitted to react over a 24 hr period at 23°C. Periodically an aliqot was taken and examined by liquid chromatography using a 500 A styragel column. A measure of the hydrolysis was the relative amount of the parent silacrown remaining at each measurement.

RESULTS

The results of the normal transport runs for potassium picrate using the silacrowns and their corresponding polyethylene glycol hydrolysis products are presented in Table 1. These data clearly indicate that both the macrocyclic polyethers and their ring opened counterparts are effective at facilitating transport. Furthermore, the data indicate that the glycols are not grossly inferior to the silacrowns in this respect since the ratios of [transport using crowns/transport using glycols] are small in these particular experiments, and never exceed an order of magnitude. The kinetic results (Table 2) of the hydrolysis of the silacrowns in saline solution clearly demonstrate the hydrolytic instability of the ionophores over a relatively short time period (24 hr), and confirmed the formation of the corresponding polyethylene glycols. This behaviour is expected for the Si-O-C linkage [2].

The graphic displays of the results of the partitioning experiments for sila-14-crown-5, and hexaethylene glycol are presented in Figs. 2 and 3. They clearly indicate the difference in partitioning between the hydrophobic phase and water for the silacrowns as compared to the glycol hydrolysis products. Over the time periods in which preequilibrium between the hydrophobic ionophore solutions and buffered aqueous

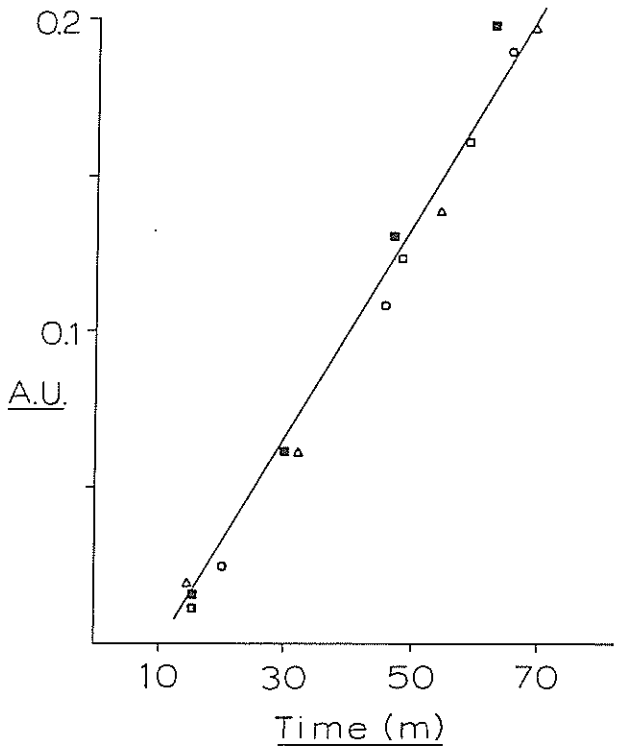

FIG. 2. Plot of absorbance in "out" arm versus time for the Sila-14-Crown-5. ■—No preequilibrium; ▽—15 m preequilibrium; ○—30 m preequilibrium; □—60 m preequilibrium.

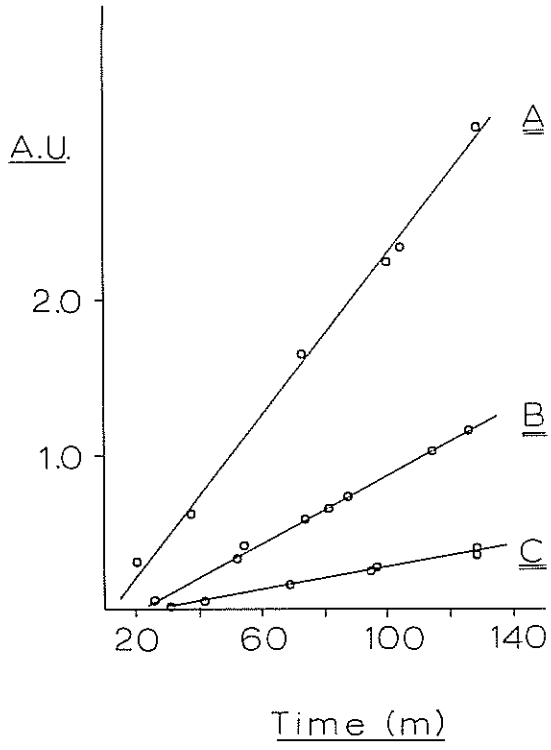

FIG. 3. Plot of absorbance in "out" arm versus time for hexaethylene glycol. A—No preequilibrium; B—15 m preequilibrium; C—30 m and 60 m preequilibrium.

solutions are occurring, the silacrowns do not partition into the aqueous phase to any detectable amount, based upon transport rates. Exactly similar results were obtained using the unsubstituted crown ether, 15-C-5, indicating that such partitioning is not solely the result of the presence of the well established hydrophobic organosilicon moeity. The glycols behave in a different manner, partitioning significantly, hence being lost upon removal of the two "in" and "out" aqueous phases. The extent of partitioning will depend upon their concentrations in the hydrophobic phase and the volume of the aqueous phases. In the present set of experiments, for the hexaethylene glycol, complete equilibration of the glycol between chloroform and water occurs after approximately 30 m, Fig. 3. No attempt has been made in this work to determine the rate of such partitioning.

DISCUSSION

We have demonstrated that the silacrown ionophores are susceptible to hydrolysis when treated with saline solution at pH 7.4, to form the corresponding polyethylene glycols. The rate at which this occurs is fast enough to suggest that in vivo the silacrowns will have a limited lifetime, prior to formation of the corresponding glycolic compound.

The comparative rate study between the silacrowns and their glycol counterparts has illustrated that there is not a great deal of difference between their efficiencies as ionophores in the type of experiment that we have performed. It is clear, and has been demonstrated by X-ray crystal structure analysis [6], that the glycols are capable of sequestering alkali metals and providing a hydrophilic environment for the cations, while at the same time presenting an external hydrophobic shield needed for entry into, and passage through, a hydrophobic region. If the toxicity of the various ionophoric substances is due to their ability to leak alkali metals across membranes why then are the silacrowns conspicuously non-toxic when compared to the normal crown ethers?

The ability of the crown ether ionophores to transport metals across membranes in vivo is predicated upon their ability to partition into the hydrophobic regions of various membranes, where they will act as carriers. This function will compete with, and disturb, the natural transport processes of the particular system with which they are involved. It is this function which presumably gives rise to their undesirable physiological properties. Since most synthetic ionophores have great thermodynamic, kinetic, and hydrolytic stability, once they have entered into the membrane hydrophobic phase they will tend to stay there for long periods of time, permitting cation leakage, hence their toxic nature. For the silacrowns which also partition into the hydrophobic phase, there is a sufficient amount of water present to permit the slow hydrolysis to the glycolic materials. As we have demonstrated, these will rapidly partition into the aqueous phase and be removed by normal excretion processes. The result is that ionophoric materials will not remain in the hydrophobic regions of membranes longer than the rate of hydrolysis of the silacrowns permit, and thus cation leakage is held to a minimum.

We are currently investigating the rate of silacrown hydrolysis in an environment such as a membrane hydrophobic phase with limited concentrations of water.

An important implication stems from the results presented in this report. It has been reported that the naturally

occurring ionophore X-537A, which is selective for calcium binding and transport, has been used as an experimental drug to change hemodynamic responses of dogs undergoing open heart surgery [8] It is a long term goal of our research to synthesize silicon based ionophores which have the high selectivity and efficiency of cation transport noted for the naturally occurring species. By building into the structures hydrolytically labile bonds hydrolysis of the ionophore over short time periods will occur to yield readily expelled fragments, and non-toxic short term drugs.

ACKNOWLEDGEMENTS

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