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INFLUENCE OF NEGATIVE ALLOSTERIC COOPERATIVITY IN CATION TRANSPORT.

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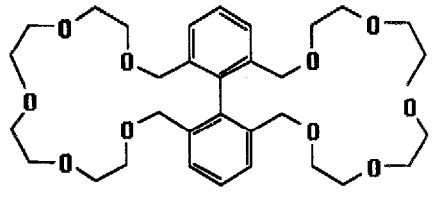
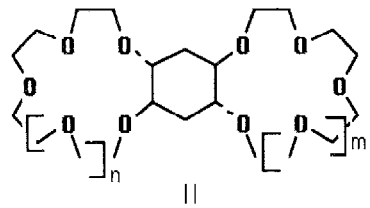
Abstract: The bis-macrocyclic ether **5** seems to have a negative allosteric cooperativity and is able to transport double the amount of Na^+ and K^+ cations as monocyclic systems. This compound could be used as a simple model of the plasma membrane Na^+-K^+ATPase which actively pumps Na^+out and K^+ into the cell, respectively.

Our latest studies on allosteric cooperativity and transport[1] show that the resulting positive cooperativity in crown ether **I** is expressed by the reduced release rate from the doubly bound crown. This behaviour could be explained because the second binding site shows greater affinity and tighter binding than the isolated subunit. A system in which binding at one site increases binding elsewhere displays positive cooperativity; the opposite result, negative cooperativity, is also possible.

The cyclohexyl system may provide the necessary rigidity for constructing the first negative cooperativity model. The simplest system would involve two binding sites arranged so that only one site at a time has a favorable conformation for binding. It may be seen that in **II**, where the dynamics of the interconversion shows results in time-averaged equivalency of the two sites, only the site with the diequatorial



I



II

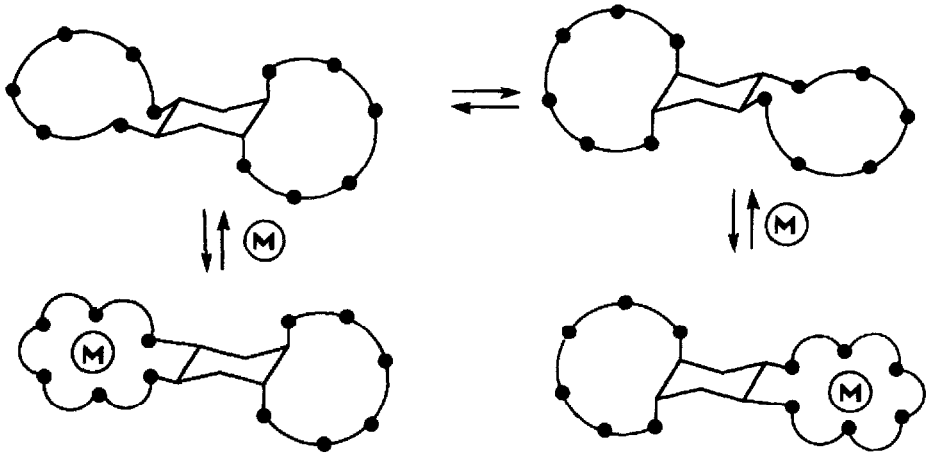
oxygens is properly arranged to converge on the metal center. Binding at the second site would tend to force out the initial guest (Scheme I).

If $m \neq n$ in **II**, then each one of the binding sites could complex a different cation. So if $m = 1$ and $n = 3$, one of the binding sites should be able to complex preferably Na^+ and the other K^+[2].

With a system such as **II**, it is possible to simultaneously transport Na^+ and K^+ across a liquid membrane, and this transport could theoretically be increased and controlled by negative cooperativity. This system seems to be useful as a model of the plasma membrane Na^+-K^+ATPase, which actively pumps Na^+ out and K^+ into the cell, respectively, to maintain cytoplasmic ion concentrations. In such a system two different ATPase conformations have been postulated[3]. The conformational change seems to be produced by the ATP participation. Compound **II** is able to suffer the conformational change only through the presence of Na^+ or K^+ions.

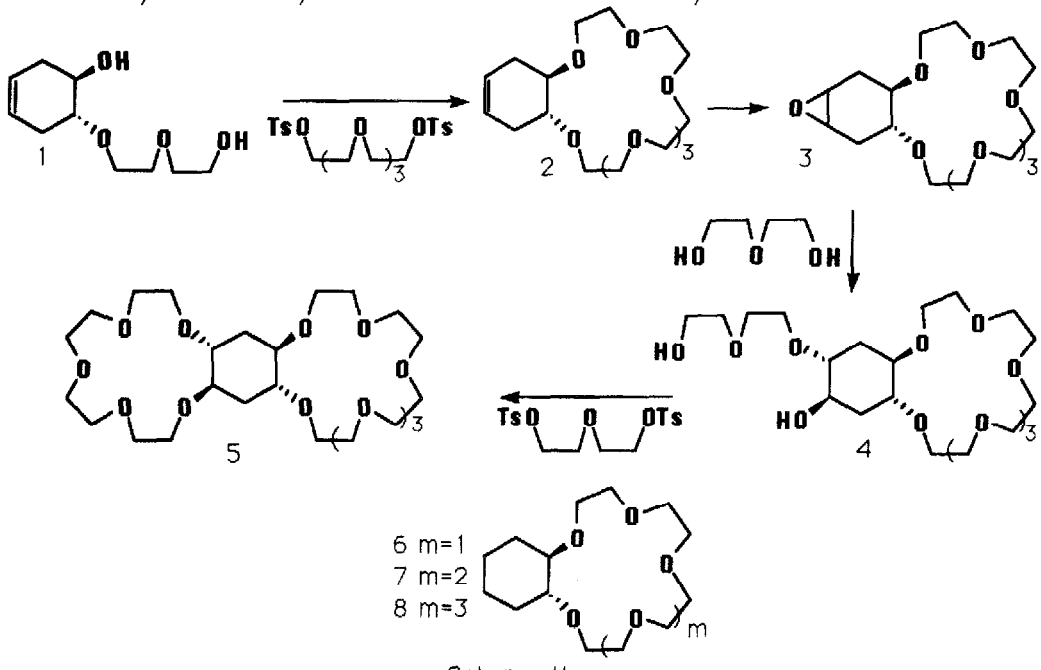
The synthesis of **5** was carried out in the following way: 1,4-cyclohexadiene was converted into its monoepoxide by reacting with hydrogen peroxide and ethyl

chloroformate[4]. The monoepoxide was opened with diethylene glycol to the trans compound **1**[5], from which the first ethereal cavity was constructed by condensation with the appropriate glycol ditosylate[6] (Scheme II). Repetition of the sequence leads to trans diaxial opening of the epoxide (**3**→**4**) to the second diol necessary for the construction of the second cavity[7].



Scheme I

In addition, the cyclic counterparts **6**, **7**, and **8** were prepared from cyclohexene by using similar procedures. These compounds had been previously synthesized[4], but several modifications were introduced into the synthesis to improve yields. A related compound was synthesized by Owen[8] but its stereochemistry was cis-cisoid-cis.



Scheme II

Extraction of aqueous solutions of alkali picrates into organic solvent was introduced by Pedersen[6] to study the complexing properties of cyclic polyethers. Qualitative determinations with our bis-cyclic and monocyclic ethers show that all of them are able to complex sodium and potassium picrates. Quantitative determination of association constants was carried out by the ultraviolet method described by Cram[9]. Results obtained are shown in Table I. It has been impossible to determine the the second association constant for the bicyclic ether as a consequence of the system stereochemistry.

Table I. Association constants determined by UV method.

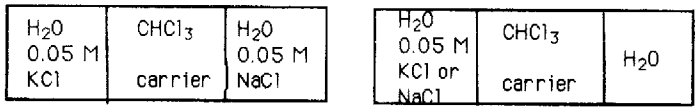
Carrier	K^+	Na^+
CH-5 (**6**)	$2.52\ 10^3$	$7.81\ 10^3$
CH-7 (**8**)	$6.49\ 10^3$	$2.45\ 10^3$
5-CH-7 (**5**)	$4.49\ 10^2$	$1.21\ 10^2$

Transport of Na^+, and K^+ across a $CHCl_3$ liquid membrane with **5** and controls **6**, **7**, and **8** was examined within a 1.3 cm diameter U-Tube at room temperature (23-25°C). The receiving phase was analysed after 24 h for Na^+ and K^+ by atomic absorption spectroscopy. Small temperature variations of a few degrees have no effect upon cation transports over a period of 24 h. In order to provided reproducible stirring of the membrane phase, synchronous motors were used to turn small magnetic stirrer bars at 120 r.p.m. The control experiments with monocyclic ethers **6-8** established that all transport Na^+ and K^+ between two different aqueous solutions separated by a chloroform phase. One of the aqueous solution is 0.05 M in NaCl and the other has the same concentration in KCl (Scheme III (A)). As is reflected in Table II, **6** preferably transports Na^+ and **7** K^+; in **8** Na^+/K^+ transport is around 1, which means that both cations are transported in similar proportions.

Table II. Transport of K^+ and Na^+ (10^{-6}mol cation/mmol carrier)

Carrier	K^+	Na^+
CH-5 (**6**)	0.404	0.681
CH-6 (**7**)	0.705	0.413
CH-7 (**8**)	0.574	0.599
5-CH-7 (**5**)	2.160	2.070

Experiments of just one cation (K^+ or Na^+) transport with bis-cyclic systems **5** (Scheme III (B)) was carried out, and in both cases cation transport was negligible, the behaviour of **5** in K^+/Na^+ transport was quite different; this carrier transported twice as much of both Na^+ and K^+ as monocyclic compounds. (Graph 1).

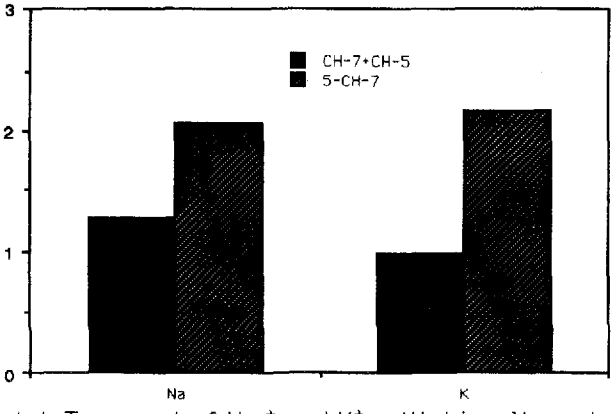


(A) (B)

Scheme III

The negative allosteric cooperativity could explain the results obtained in the one cation transport experiments; the two-site systems provide a cation transport negligible in comparison to those of the two corresponding monocyclic together (on a per site basis). These results are in accordance with theory, because the negative allosteric cooperativity prevents the second complex formation.

In the two cation experiments the situation is completely different. **5** presents an odd behaviour; it is able to transport twice as much as the two corresponding monocyclic compounds. One explanation for this behaviour could come from the negative cooperativity shown by **5**. When the bis-cyclic ether carrying K^+ ions arrives at the NaCl solution across the liquid membrane, the presence of Na^+ forces the conformational change and K^+ is released more easily, thus also increasing transport. Compound **5** is an adequate model for the Na^+-K^+ pump because a conformational change controls the ion transport.



Graph I. Transport of Na^+ and K^+ with bicyclic and monocyclic ethers

In conclusion, we have prepared a system which seems to show negative allosteric cooperativity. In this kind of system the single subunit crown ether complex formation forces the two oxygens to remain in the diequatorial conformation. This conformation is transmitted through the cyclohexane to the second crown ether subunit. Consequently, this second subunit has its two oxygens in diaxial conformation and complex formation is hindered.

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References

1. a) Gaviña, F.; Luis, S.V.; Costero, A.M.; Burguete, M.I.; Rebek, J. Jr. *J. Am. Chem. Soc.* **1988**, *110*, 7140-7143. b) Rebek J.Jr.; Wattley R.V. *J. Am. Chem. Soc.* **1980**, *102*, 4853-4854
2. Frensdorff, H.K., *J. Am. Chem. Soc.*, **1971**, *93*, 600-606.
3. Bach, R.D.; Klein, M.W.; Ryntz, R.A.; Holubka, J.W. *J. Org. Chem.* **1979**, *44*, 2569-2571
4. Samoshiv, U.V.; Zelenkina, O.A.; Aubbotin, O.A. *Zh. Org. Khim.* **1988**, *24*, 465-471
5. Chapoteau, E.; Czech, B.P.; Kumar, A.; Pose, A. *J. Org. Chem.* **1989**, *54*, 861-867
6. Pedersen, C.J. *J. Am. Chem. Soc.* **1969**, *92*, 391-394
7. 21% yield, ^1H NMR (200 MHz, $CDCl_3$)0.9-1.15 (m, 1H); 1.7 (m, 1H); 3.3-3.6 (m, 22H). ^{13}C NMR (200 MHz, $CDCl_3$) 70.893 (broad). Anal Calcd for $C_{26}H_{48}O_{12}$: C56.52%; H, 8.69%. Found C,56.26%; H, 8.91%. The trans-transoid-trans stereochemistry of this compound has been determined by using Eu (hfc)3 because 5 is a chiral compound.
8. Owen, J.D. *J. Chem. Soc. Perkin Trans II*, **1983**, 407-415
9. Moore, S.S.; Tarnowski, T.L.; Newcomb, M.; Cram, D.J. *J. Am. Chem. Soc.* **1977**, *99*, 6398-6405

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