Membrane Transport. A Proton-driven Potassium Ion Pump

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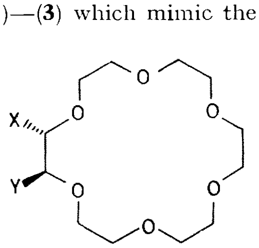
Summary The functionalized crown ethers (**1**)—(**3**) act as carriers for the membrane transport of potassium ions against their concentration gradient by the coupled counter-transport of protons.

THE membrane transport of cations, mediated by ionophore antibiotics[1] and synthetic carriers[2] is well established. In particular, antibiotics of the nigericin group[1] mediate the coupled counter-transport of cations and protons, and this property has been exploited in an artificial sodium ion pump.[3] We report here the synthesis and transport properties of some macrocyclic polyether derivatives (crown ethers) (**1**)—(**3**) which mimic the counter-transport behaviour of the nigericin antibiotics. These model systems are susceptible to systematic structural modification[4] and should thus permit a detailed examination of the influence of carrier structure on transport. In addition to providing insight into natural systems, these studies may lead to important practical applications.



(**1**) X,Y = CO_2H, $CONH[CH_2]_7Me$
(**2**) X,Y = CO_2H, $CONH[CH_2]_{13}Me$
(**3**) X,Y = CO_2H, $CONH[CH_2]_{17}Me$
(**4**) X = Y = $CONMe_2$
(**5**) X = Y = CO_2H
(**6**) X,Y = -CO-O-CO-

The carriers (**1**)—(**3**) were prepared from the bisdimethylamide of (+)-tartaric acid and 1,14-di-iodo-3,6,9,12-tetraoxatetradecane using the thallium alkoxide cyclization method[5] to give the tartramide crown ether (**4**) (yield 40%). Acidic hydrolysis yielded the diacid (**5**) (75% yield) which was converted into the anhydride (**6**) by treatment with acetyl chloride. When treated with an excess of octylamine, tetradecylamine or octadecylamine, (**6**) gave the carriers (**1**)—(**3**) as their respective alkylammonium salts. Ion exchange and reverse-phase chromatography gave the free carriers (**1**)—(**3**) as viscous oils [yield 70% from (**5**)].

The transport of potassium ions across a chloroform membrane was studied using a U-tube apparatus similar to those previously described.[2] The carrier in chloroform was placed in the base of the U and buffered aqueous solutions of equal potassium ion concentration were placed in the arms of the U, floating on the chloroform. Buffer solutions were selected to impose a constant proton gradient across the membrane. Potassium was transported from the basic to the acidic side of the membrane thereby creating a potassium ion gradient. Typical curves showing potassium concentration as a function of time are given in the Figure. Two types of curves are reproduced in the Figure: a case where the potassium concentration is much greater than the carrier concentration (A/A′) and a case where the amounts of carrier and potassium ion transported are comparable (B/B′). In the former case, the concentration of the potassium complex in the membrane reaches a steady state. Both the amount of potassium lost from the

TABLE. Initial concentrations and maximum transport rates of potassium ions by carriers (**1**), (**2**), and (**3**).[a]

Entry	Carrier	10^3 [Carrier]/M	Initial 10^3 [potassium]/M	pH[b] basic side	pH[c] acidic side	Type[d]	10^6 × Maximum[e] transport rate/ (mol h^{-1})
1	(**1**)	1·67	1·0	8·56	2·30	B	1·36
2	(**2**)	1·37	1·0	8·56	2·30	B	2·48
3	(**3**)	1·48	1·0	8·56	2·30	B	2·53
4	(**1**)	0·65	5·0	8·56	2·30	A	0·76
5	(**2**)	0·48	5·0	8·56	2·30	A	1·59
6	(**3**)	0·67	5·0	8·56	2·30	A	1·69
7	(**2**)	1·15	1·0	8·56	1·40	B	2·26
8	(**2**)	1·15	1·0	8·56	2·30	B	2·25
9	(**2**)	1·15	1·0	8·56	3·35	B	2·35
10	(**2**)	0·55	1·0	8·56	2·30	A	0·95
11	(**2**)	0·55	5·0	8·56	2·30	A	1·68
12	(**2**)	0·55	10·0	8·56	2·30	A	1·86
13	(**2**)	0·55	5·0	10·54	2·30	A	1·53
14	(**2**)	0·55	5·0	11·81	2·30	A	1·68

[a] Rates at 25 ± 0·2 °C for a U-tube apparatus of 15 mm i.d. tubing, 20 mm radius bend. The jacketted cell contained two 10 ml aqueous phases in the arms of the U floating on 12 ml of chloroform containing the carrier. The interfaces were located in the vertical sections of the U and had areas of 1·54 cm^2. All phases were stirred at 150 Hz. [b] Basic buffer solutions: pH 8·56, tris(hydroxymethyl)aminomethane (Tris)–Tris·HCl: [Tris] = 0·25 M except entries 1—3, where [Tris] = 0·05 M; pH 10·54, glycine–tetramethylammonium glycinate, [glycine] = 0·25 M; pH 11·81, 0·01 M tetramethylammonium hydroxide. [c] Acidic buffer solutions: pH 1·40, 0·25 M phosphoric acid; pH 2·30 phosphoric acid–tetramethylammonium dihydrogen phosphate, [phosphate] = 0·25 M except entries 1—3 where [phosphate] = 0·05 M; pH 3·35, phosphoric acid–tetramethylammonium dihydrogen phosphate, [phosphate] = 0·5 M. [d] The type of curve observed for a plot of potassium ion concentration *vs.* time. Type A: linear with time, see A,A′ in Figure; type B: consecutive first-order reaction type, see B,B′ in Figure. [e] Calculated as described in the text. The errors in the maximum transport rates are *ca.* ±10% as assessed from the accuracy and reproducibility of the experiments.

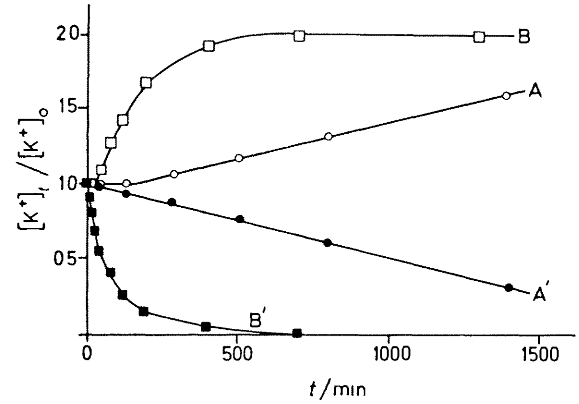


FIGURE Variation in potassium ion concentration as a function of time The vertical axis is the ratio of the potassium ion concentration at a given time to the initial potassium ion concentration This permits the two curves differing by a factor of five to be presented on a common axis Curve A, ○, acidic side entry 13 curve A' ● basic side entry 13 curve B, □, acidic side entry 9 curve B' ■ basic side entry 9

basic side, and, after a brief induction period, the amount of potassium gained on the acidic side of the cell increased linearly with time

The case represented by curves B and B′ is more complex The kinetic behaviour can be treated as an example of two consecutive, irreversible first-order reactions Standard graphical[6] and iterative techniques gave a pair of first-order rate constants and the solid lines of curves B/B′ are calculated from these values In order to compare the two types of behaviour we have calculated the maximum rate at which potassium ions are released to the acidic side of the membrane This is the slope of the linear portion of curve A or it can be calculated from a knowledge of the two rate constants necessary to reproduce curve B [6] The data from these analyses for varying carrier type and concentration, potassium concentration, and acidity are given in the Table

A number of points may be noted First, the acidity of neither the right- nor left-hand aqueous phase plays a significant role in the rate of potassium transport (entries 7—9 and 11, 13, 14) This is in contrast with the analysis of Cussler and his co-workers[3] who predicted, and found a dependence of 1/rate on $1/[M^+][OH^-]$ for the basic side of the membrane However, as predicted,[3] we observe a direct dependence of transport rate on carrier concentration, doubling of carrier concentration roughly doubles the rate (*e g* entries 2 and 10) The dependence on potassium ion concentration is less marked, a tenfold increase leads to only a doubling of the rate (*e g* entries 10 and 12) The exact form of these functions awaits further experiments at much higher carrier and potassium concentrations

Of the three crown ether derivatives synthesized, (**1**), bearing an octyl side chain is clearly inferior to (**2**) or (**3**) (entries 1—6) Although (**3**) extracts potassium much more efficiently than (**2**), the release rate is slower such that the *net* transport rate reflected by the maximal rate of release is similar for the two compounds These effects are doubtless due in part to differing lipophilicities of the different compounds and detailed investigations of the exchange reactions involved are in progress to elucidate the structural origins of these differences

Finally, that counter-transport of potassium ions and protons has occurred can be shown by measurement of the final pH values for the two aqueous phases For example, for experiment 2, on the acidic side the pH increased by *ca* 0 15 units, on the basic side the pH *decreased* by the same amount From the buffer concentration (0 05 M) we calculate these observed pH changes to correspond to the trasnfer of *ca* 1×10^{-5} mol of protons from right to left The potassium concentration data indicate a transfer of the same quantity of potassium from left to right Counter-transport has thus occurred

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