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Allosteric Behaviour of a Bis-cyclic Crown Ether Derived from Biphenyl

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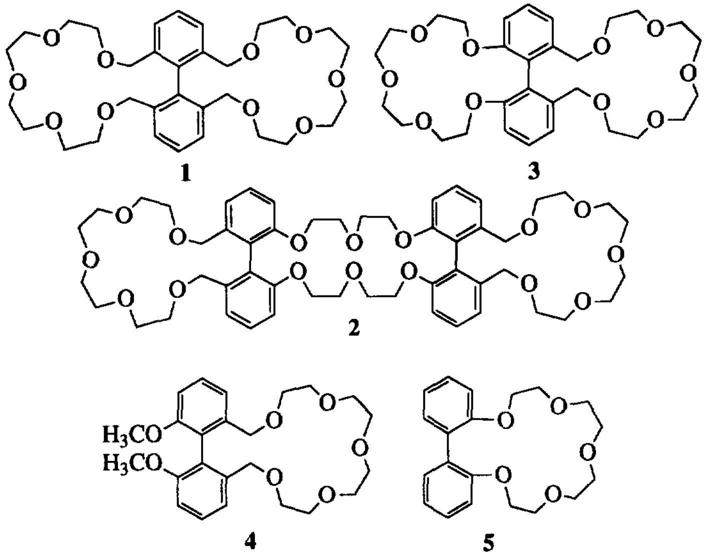
Abstract: Complexation and transport studies carried out using bis-cyclic crown ether derived from biphenyl have demonstrated that compound **3** shows a negative allosteric cooperativity in Na^+ transport when $Hg(SCN)_2$ is present in the solution. Copyright © 1996 Published by Elsevier Science Ltd

Compounds showing allosteric behaviour have been widely studied in order to use this characteristic for increasing transport ability across liquid membranes. Since 1981, when Rebek[1] synthesized compound **1**, the rigidity of the biphenyl system has been used for transmitting conformational information between complexing subunits. Subsequent experiments carried out with this compound showed that positive allosteric cooperativity does not always provide a most efficient transport of cations across liquid membranes.[2] In this case, the resulting positive cooperativity is expressed in the reduced release rate from the doubly bound crown. This behaviour is produced because the second binding site of **1** shows greater affinity and tighter binding than the isolated subunit.

In order to increase transport efficacy other compounds have been prepared. Thus, our research group prepared compound **2**.[3] In this compound, one of the three subunits, the central one, is different not only in its size but in the characteristics of donor atoms as well. In addition, the three crown ethers are conformationally related through the two biphenyl systems. Compound **2** presents positive allosteric cooperativity between the two external subunits, but the central crown ether has a conformation which is not suitable for complexing the metal species. Thus, when carrier **2** is used to transport $Hg(CN)_2$ across liquid membranes, only one external subunits is involved in transport and the other external solely acts as an activator homotropic centre.

According to these previous experiments, our research group prepared compound **3** in which there are two complexation sites with different sizes and electronic characteristics.[4] This compound complexed $Hg(SCN)_2$ giving the 1:1 complex, and the corresponding association constant was determined by using NMR (83 M^{-1}, acetone 25°C). Complexation behaviour of compound **3** was compared with that observed in **4** that is an adequate model for the benzylic subunit of **3**.

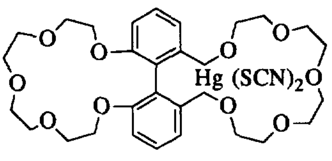


Scheme 1

Now we report a study about the allosteric cooperativity of compound **3**, and its behaviour in transport experiments. As appropriate models, compounds **4**[3] and **5** were used. Compound **5** was prepared from 2,2-biphenol and tetraethyleneglycol ditosylate under standard conditions[5].

COMPLEXATION EXPERIMENTS

Mercury derivatives._ The syntheses of the 1:1 complexes of **3** and **4** with $Hg(SCN)_2$ and the crystallographic structure of **4**·$Hg(SCN)_2$ had been previously described by our research group,[3,4,6] but as compound **3** is asymmetric it was necessary to determine in which cavity the metal was complexed. X-ray diffraction experiments show that in **6** the mercury atom is complexed by the benzylic subunit.



6

The comparison of this structure with that observed in **4**·$Hg(SCN)_2$ proves that in both complexes all the etheral atoms participate in the coordination of the mercury. In addition, in the former, the oxygen atoms in the ring are, on overage, 0.223 Å from the oxygen mean plane, and the oxygens do not alternate above and below this mean plane. In the complex, the mercury atom is lying at aproximately the oxygen mean plane and occuping a central position within the cavity. On the other hand, the Hg-S bond length is 2.363 Å which accords with the standard values.[7] The Hg-O coordination length is unexceptional at 2.816 Å. Thus, in the complex, the Hg atom is coordinated equatorially by five oxygen atoms resulting in coordination polyhedra best described as distorted pentagonal bipyramidal that involves both sulphur atoms. In conclusion, there are not big differences between both structures and it seems that the presence of the second crown moiety has not influence on the geometry of the complex.

In respect of the phenolic subunit, some interesting conclusions could be obtained based on the X-ray data. Thus, the 17-membered ring in **6** is much smaller than does the 19-membered cavity, and the mercury atom is too big to be complexed. The same behaviour was observed in compound **5** that had never complexed $Hg(SCN)_2$ even though conditions (solvent, temperature and time) had been widely changed.

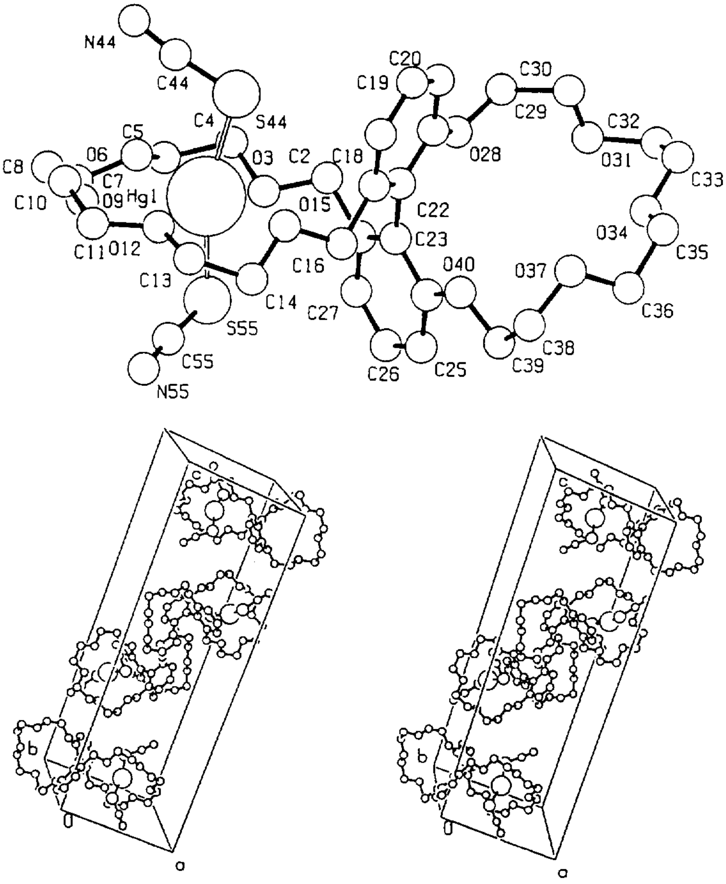


Figure 1. Molecular structure with crystallographic numbering scheme and stereoscopic view of its crystal packing of complex **6**. Hydrogen atoms have been excluded for clarity.

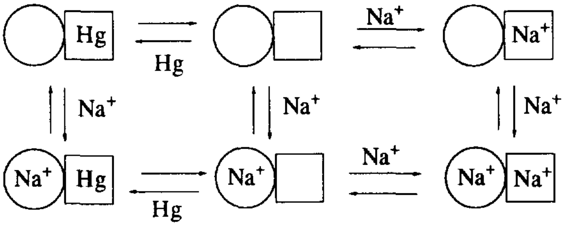
On the other hand, a distortion of the 1,4-dioxa units of the pentaether is observed in this phenolic subunit. In the first anisotropic refinement process, an inusual short C(30)-O(31) length (1.085 Å) was obtained. In order to improve the results taking into account a chemical point of view, a DFIX instruction of the SHELXL-93 program was carried out with a fixed length C(30)-O(31) of 1.42 ± 0.005. Under these conditions, temperature factors and length of these atoms improve. However, a distortion in this part of the molecule persists what is revealed by angles from O(28) to C(33) that has values far from the standard. In consequence, geometry of the phenolic crown ether in complex **6** only allows oxygens O(31), O(34), and O(37) to present a suitable arrangement for complexing other cation; whereas, oxygens O(28) and O(40) have their lone pairs directed outside the cavity. Thus, the presence of $Hg(SCN)_2$ in the benzylic crown ether has a strong influence on the geometry and complexing ability of the phenolic subunit.

Finally, experiments carried out in solution, using acetone as solvent allowed to determine the value of the association constant. However, as a consequence of the reason exposed below, the 1:2 complex with **3** was never obtained even when the added amount of $Hg(SCN)_2$ was higher than 1 equiv. Under these conditions the signals corresponding to the ligand decreased until vanishing, according with Le Chatelier's principle. In conclusion, it is possible to affirm that compound **3** is not able to present allosteric cooperativity because of the small size of the phenolic cavity.

When the experiment was carried out in chloroform only **6** was present in the solution, that is according to the smaller solvent polarity. Furthermore, similar behaviour than before was observed in this solvent.

Alkali cations._ The complexation of sodium picrate in acetone was carried out using **3**, **4**, and **5**. With alkali metals, exchange is usually rapid, and the weighted average of free and bound species is observed. In spite of that, plots of $\Delta\delta$ *vs.* amounts of Na^+ added allowed to determine the value of association constants of **4** and **5** in acetone, and they were 96 M^{-1} and 26 M^{-1}, respectively.[8] However, in the case of the bis-cyclic compound **3**, where three rapidly equilibrating species were present in the presence of Na^+, non linear least-square regression analysis of the data was required and the values of the constants were uncertain. Similar attemps to bind alkali metals were made by Rebek[9] and Laszlo[10] with parallel conclusions.

More interesting results were obtained when **6** was used as a ligand for complexing Na^+. In this case, all the equilibria that could be established are these reflected in the scheme 2.



Scheme 2

Before the addition of sodium picrate will start, two species (the free ligand and the complex) were present, and their signals were observed in a separate way in the NMR spectrum. Na^+ addition allowed to observe a shift for the signals of both species what means that there are present new complexes. First of all, the complex with Hg in the benzylic subunit and Na^+ in the phenolic one will be present. In addition, complexes between **3** and sodium picrate with 1:1 or 1:2 stoichiometry will be also in solution. In the complexes with the free ligand **3** what is really observed is the equilibrium between all these species, and therefore, it is impossible to determine the corresponding association constants. Moreover, the association constant of **6** with Hg $(SCN)_2$ neither could be determined, because the amount of Na^+ added was not known as a consequence of that Na^+ ions were also complexed by the free ligand.

On the other hand, the relationship [ligand] / [complex], measured by using the integral values in the NMR spectra, decrease as the amount of picrate increased. This fact could be explaining taking into account that formation of **6**·NaPic complex shifted the equilibrium $\mathbf{3}\rightleftarrows\mathbf{6}$ toward the mercury complex.

When an excess of $Hg(SCN)_2$ was added to a solution of **6** in acetone, only the signals corresponding to **6** were observed, what is according to the Le Chatelier principle. Under these conditions titration experiments allowed us to know the association constant of the phenolic subunit in **6**. The value of this constant was 19 M^{-1} which is slightly smaller than that observed for **5**. However, this difference is too small to conclude anything about allosteric cooperativity, and it could be due to other factors like lipophilic character, preorganization or solvation.

TRANSPORT EXPERIMENTS

Transport of NaPic through a chloroform membrane was examined within a U-tube. An aqueous solution of sodium picrate was placed in the left arm of the tube and UV spectroscopy was used to determine the transport to the right arm. **3** and **6** were used as carriers for transporting the alkali cation, and control experiments were carried out by using the monocyclic compounds **4** and **5**. The results obtained in these experiments are reflected in the Table 1.

First of all, two comparative experiments were carried out by using different carriers. In the first one, an equimolecular mixture of **4** and **5** was employed, and in the second one, the carrier was an equimolecular mixture of **4**•$Hg(SCN)_2$ and **5**. An NMR study of the organic phase in this second experiment demonstrated that the mercury atom remained in the benzylic crown ether even in presence of large quantities of sodium picrate. On the other hand, a comparison of the values reflected in the Table 1 (run 1 and 2) allowed to establish that **5** is 24.5% less effective in transporting the cation across the liquid membrane than the benzylic compound **4**.

Table 1

run	carrier	$[NaPic]_{\text{receiving phase}}$ M	Efficacy (mmol/mmolhole)
1	**4+5**	$1.825\ 10^{-3}$	51.4
2	**4**.$Hg(SCN)_2$ +**5**	$7{,}851\ 10^{-4}$	44.4
3	**3**	$1.160\ 10^{-3}$	33.3
4	**6**	$1.375\ 10^{-4}$	7.9

When **3** was used as carrier, only $5.82\ 10^{-4}$ g. of sodium picrate were transported (run 3) which proves that the efficacy of **3** in transport is lower than that observed for an equimolecular mixture of **4** and **5**. Although several reasons could have influence on this behaviour, probably, the Coulombic repulsion is the strongest factor for decreasing Na^+ transport across the membrane. These electronic repulsions make binding of a second ion more difficult than the first. Thus, the influence of the complexation in the first cavity on the ability of the second crown to bind the cation can not be determined.

Therefore, in order to determine the allosteric behaviour of both cavities, **6** had to be used as carrier. In this compound, the mercury atom is complexed in the benzylic subunit, and the phenolic cavity is available to transport sodium picrate. This fact was confirmed by studying the NMR of the chloroform solution where only the species with Hg into the benzylic cavity was detected. In this way, it is possible to study the influence of the first complex in the ability of the second subunit for binding the alkali cation by comparing run 2 and 4 in the Table 1. Taking into account the covalent characteristics of $Hg(SCN)_2$, no Coulombic repulsions are expecting in this case. However, **5** transport six times more sodium picrate than the phenolic cavity of **6**. This behaviour only could be explained if the formation of the mercury complex fixes the conformation of the phenolic unit in such a way that it is unsuitable for complexing the cation. Thus, the bis-cyclic system showed in this experiment negative allosteric cooperativity.

This assumption accords with the conformation observed by using CPK models. On the other hand, molecular mechanics calculation showed for ligand **3** a high symmetry for all the ethyleneoxy units in the phenolic cavity, whereas the X-ray data of **6** allowed to verify that a strong distortion is produce in the cavity when the mercury atom is complexed by the benzylic crown.

CONCLUSIONS

The results obtained in the experiments previously described allow to establish several conclusions. First of all, the phenolic cavity of compound **3** is too small to complex mercury and for this reason the 1:2 complex has never been observed. In addition, when the mercury atom is complexed by the benzylic cavity, the conformational change induced on the phenolic cavity make two of the five oxygens in this subunit unable to bind new species. What is more, there are not strong differences between the complex geometry in solution and in the solid state because transport across liquid membranes with this carrier, **6**, demonstrated a small ability in complexing NaPic. Finally, as a consequence of the geometry, **6** showed negative allosteric cooperativity which was reflected in the amounts of cation transported.

EXPERIMENTAL SECTION

NMR spectra were recorded on a Bruker AC-250, Varian Unity-300 and 400 spectrometers. Chemical shifts were reported in parts per million downfield from TMS. Residual solvent o TMS was taken as a reference. UV spectra were taken on a Shimadzu uv-2101/3101PC spectrometer.

Compounds **3**, **4** and **5** were obtained as has been previously described in the literature[3,4,5]. All them were fully characterized.

X-Ray Structure Analysis

Information concerning crystallographic data collection and refinement for complex **6** are summarized in Table 2. Intensity measurements were made on an Enraf-Nonius CAD4 diffractometer using a single crystal of dimensions 0.15 x 0.05 x 0.12 mm. Graphite-monochromated Mo-Kα radiation (λ = 0.71069 Å) and ω-scan technique was used. Data collection were carried out at room temperature. Three reference reflections were measured every 2 hours as intensity and orientation check and no significant fluctuation was noticed during the collection of the data. Lorentz-polarization and empirical absorption (Ψ scans) corrections were made. The crystal structure was solved by Patterson methods[11] using the SHELXS86 program and refined by full-matrix least-square techniques[12] on F^2. The non-hydrogen atoms were refined anisotropically. Nineteen of the forty-two hydrogen atoms of the asymmetric unit were found in the Fourier difference-synthesis and the rest of them were geometrically constructed with the SHELXL93-Program with fixed isotropic displacement parameters. Fractional atomic coordinates with standard deviations (in parentheses) and equivalent isotropic temperature factors U(eq) are shown in Table 3.

The plots (Figure 1) for the molecule and its packing in the unit cell were made with PLUTON[13]. Geometrical calculations were performed by using PARST[14]. A list of anisotropic displacement parameters for non-hydrogen atoms, bond lengths and angles, torsion angles, hydrogen atoms coordinates and tables of calculated and observed structure factors (F_o-F_c) have been deposited as supplementary material at the Cambridge Crystallographic Data Centre.

Table 2. Crystallographic Data and Structure Refinement for Complex **6**.

Empirical formula	$C_{34}H_{42}O_{10}HgN_2S_2$	
Formula weight	791.28	
Temperature	293(2)K	
Wavelength	0.71069 Å	
Crystal system	Monoclinic	
Space group	$P\ 2_1/n$	
Unit cell dimensions	a = 11.386(4) Å	alpha = 90.0°
	b = 9.772(1) Å	beta = 98.08(2)°
	c = 32.737(12) Å	gamma = 90.0°
Volume	3606(2) $Å^3$	
Z	4	
Density (calculated)	1.620 Mg/m^3	
Absorption coefficient	4.439 mm^{-1}	
F(000)	1760.0	
Crystal size	0.15 x 0.05 x 0.12 mm.	
Theta range for data collection	1.26 to 24.98 deg.	
Index ranges	-13<=h<=13, -11<=k<=0, -38<=l<=0	
Reflections collected	6453	
Independent reflections	6337 [R(int) = 0.0212]	
Absorption correction	Semi-empirical from psi-scans	
Max. and min. transmission	0.999, 0.640	
Refinement method	Full-matrix least-square on F^2	
Data / restrains / parameters	6335 / 1 / 500	
Goodness-of-fit on F^2	1.136	

Final R indices [I>2sigma(I)]	R(F) = 0.0510, wR(F^2) = 0.0959
R indices (all data)	R(F) = 0.1057, wR(F^2) = 0.1276
Largest diff. peak and hole	1.727 and -1.347 e.$Å^{-3}$

Table 3. Atomic coordinates (x 10^{-4}) and equivalent isotropic displacement parameters (A^{-2} x 10^{-3}) for **6**. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
Hg (1)	3847 (1)	181 (1)	1444 (1)	56 (1)
S (44)	4008 (3)	2190 (3)	1850 (1)	76 (1)
C (44)	5024 (14)	1753 (15)	2224 (6)	103 (5)
N (44)	5739 (16)	1557 (17)	2515 (4)	157 (7)
S (55)	3051 (3)	-1693 (3)	1039 (1)	76 (1)
C (55)	3997 (11)	-1803 (12)	699 (3)	65 (3)
N (55)	4594 (11)	-1897 (11)	447 (3)	87 (3)
C (1)	294 (9)	-76 (13)	1338 (3)	62 (3)
C (2)	927 (10)	2 (13)	1779 (3)	68 (3)
O (3)	1852 (6)	-951 (8)	1833 (2)	64 (2)
C (4)	2346 (13)	-1095 (16)	2249 (4)	79 (4)
C (5)	3346 (14)	-2104 (17)	2293 (5)	89 (5)
O (6)	4316 (7)	-1509 (8)	2136 (2)	74 (2)
C (7)	5227 (16)	-2428 (18)	2079 (6)	113 (6)
C (8)	6277 (13)	-1678 (18)	1977 (4)	104 (5)
O (9)	5969 (7)	-1043 (10)	1576 (2)	81 (2)
C (10)	6849 (11)	-113 (21)	1489 (4)	94 (5)
C (11)	6607 (10)	332 (16)	1053 (4)	85 (4)
O (12)	5427 (6)	864 (8)	968 (2)	67 (2)
C (13)	5189 (11)	1497 (14)	579 (4)	69 (3)
C (14)	3884 (10)	1596 (12)	451 (3)	61 (3)
O (15)	3367 (6)	2272 (8)	767 (2)	63 (2)
C (16)	2116 (11)	2267 (14)	680 (3)	60 (3)
C (17)	1608 (9)	2999 (11)	1025 (3)	53 (3)
C (18)	2047 (11)	4279 (12)	1149 (3)	59 (3)
C (19)	1576 (11)	4987 (13)	1451 (4)	74 (3)
C (20)	667 (11)	4469 (12)	1629 (3)	68 (3)
C (21)	214 (9)	3220 (12)	1507 (3)	61 (3)
C (22)	685 (9)	2434 (11)	1211 (3)	53 (2)
C (23)	164 (8)	1076 (11)	1079 (3)	51 (3)
C (24)	-458 (9)	942 (13)	674 (3)	61 (3)
C (25)	-903 (10)	-313 (15)	540 (4)	69 (3)
C (26)	-746 (11)	-1418 (16)	791 (5)	77 (4)
C (27)	-170 (10)	-1324 (14)	1187 (4)	69 (3)

O (28)	-684 (7)	2574 (9)	1677 (2)	79 (2)
C (29)	-1418 (14)	3316 (16)	1905 (5)	103 (5)
C (30)	-2165 (21)	4444 (22)	1706 (7)	211 (11)
O (31)	-3090 (14)	3636 (16)	1501 (4)	168 (5)
C (32)	-4128 (16)	4473 (17)	1345 (5)	124 (6)
C (33)	-4022 (16)	5428 (16)	1005 (5)	116 (5)
O (34)	-3530 (10)	4735 (10)	685 (3)	109 (3)
C (35)	-3397 (15)	5532 (15)	349 (5)	107 (5)
C (36)	-2913 (13)	4700 (17)	26 (5)	88 (4)
O (37)	-1682 (8)	4620 (11)	125 (3)	94 (3)
C (38)	-1206 (12)	3541 (17)	-108 (4)	91 (4)
C (39)	-1279 (11)	2124 (15)	61 (4)	80 (4)
O (40)	-551 (7)	2099 (9)	446 (2)	76 (2)

Association Constants of the Complexes with Sodium Picrate Determination. General Procedure.

Titrations of **4** and **5** were made in acetone-d_6 at constant host concentration: Aliquots of a guest solution in acetone (50µM) were added to a solution of the host (20mmol) in acetone (0.6ml) The solvent level was kept constant in every moment by evaporation and the spectra were recorded at 298K. The resulting curves obtained by following the chemical shift of the benzylic (**4**) or $-CH_2-$ linked to the phenolic oxygen signals (**5**) with the variable guest concentration were fitted by using a nonlinear least-square regression analysis.

Titration of **6** with sodium picrate in acetone-d_6 was made in a similar way, but an excess of 4 equivalents of $Hg(SCN)_2$ had to be used to shift the equilibrium toward the mercury complex in the acetone solution. The chemical shift of the $-CH_2$-linked to the phenolic oxygen was used in the association constant determination.

Transport of Sodium Picrate Through a Liquid Membrane

A glass U-tube was employed as cell for the transport experiments. The organic layer was a solution of the carrier in chloroform (6ml). The source phase was an aqueous sodium picrate solution (0.05M, 2ml), and the receiving one was made with deionized water (2ml). The contact surface between the phases (inner area) was 1.568 cm^2. The transport experiments were run during 1.5 h and throughout the stirring was keep constant (500 rpm).The amount of sodium picrate transported was monitored measuring the absorbance at 355 nm in the receiving phase at the end of the experiment. Previously the ε for this salt was determined at this wavelength (14810 $cm^{-1}M^{-1}$). Each experiment was repeated at least 3 times and the reported results are the average of the determination. The standard deviation from the mean value among the data in each experiment was lower than 5%.

Five experiments were done. In all them the conditions were the same, but only the carrier was changed:

1.- A mixture of **4** and **5** was used as carrier. The concentration in every one was 0.0029M (the concentration of holes was 0.0058M).

2.- A mixture of **4.Hg(SCN)$_2$** (0.0029M) and **5** (0.0029M) was used as carrier (concentration of phenolic holes 0.0029M).

3.- **3** (0.0029M) was used as carrier (the concentration of holes was 0.0058M).

4.- **6** (0.029M) was used as carrier (concentration of phenolic holes 0.0029M).

5.- None carrier was used in order to know the passive transport.

After the experiments 2 and 4 the chloroformic phase was evaporated and the NMR analysis showed that the mercury complex was intact.

The results are shown in Table 1. The efficiency was detemined as mmol NaPic transported/mmolhole.

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