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Dopamine-Selective Response in Membrane Potential by Homooxacalix[3]arene Triether Host Incorporated in PVC Liquid Membrane

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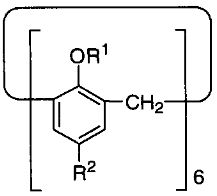
Abstract: Selectivities of membrane potential changes for catecholamines and inorganic cations were investigated with lipophilic derivatives of calix[6]arene and related hosts incorporated in poly(vinyl chloride) (PVC) matrix liquid membranes. Homooxacalix[3]arene triether displayed an excellent selectivity for dopamine against other catecholamines (adrenaline, noradrenaline) and also against inorganic cations (K^+, Na^+). © 1999 Elsevier Science Ltd. All rights reserved.

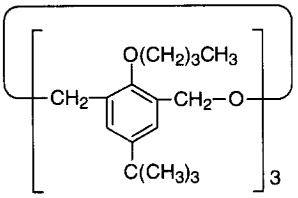
Keywords: Complexation; membranes; neurotransmitters; polycyclic; heterocyclic compounds.

Regulation of membrane potentials based on host-guest complexation is focusing increasing attention from both fundamental and practical viewpoints. So far, in context with the development of inorganic cation-selective electrodes for analytical use, studies on guest-induced changes in membrane potential (potentiometric responses) have been studied mainly with crown ethers and related hosts incorporated in poly(vinyl chloride) (PVC) matrix liquid membranes.[1a-f] In contrast, there is still a limited number of studies on potentiometric discrimination of *organic* ions by synthetic hosts.[1e,f] We have reported the first potentiometric discrimination of *nonpolar* structures of organic ions by a PVC liquid membrane incorporated with per-*O*-acetic acid ester of calix[6]arene (**1a**).[2] For this membrane, potentiometric selectivities to nonbranched primary amines (protonated form) such as 2-phenylethylamine were observed, which could be ascribed to the formation of inclusion complexes with tripodal hydrogen bonding between the $-NH_3^+$ group of the guest and the ester carbonyl oxygens of the host.

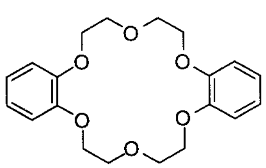
From a bioanalytical viewpoint, an interesting feature of this host is the selectivity to dopamine against other catecholamine neurotransmitters (adrenaline, noradrenaline). However, calix[6]arene hexaester **1a** still suffered from severe interference by K^+ ion. In this study, we report our efforts to obtain a prototype of calix[6]arene analogue with a high dopamine/K^+ selectivity for the development of a dopamine sensor for use under physiological conditions. For this purpose, comprehensive investigations were made on the potentiometric selectivities of several lipophilic derivatives of calix[6]arene and related hosts (**1a~d**, **2**, **3**) incorporated in PVC liquid membranes. A high dopamine selectivity was attained by the homooxacalix[3]arene triether host (**2**).



1a: $R^1 = -CH_2COO(CH_2)_9CH_3$, $R^2 = -H$
1b: $R^1 = -CH_2COOCH_2CH_3$, $R^2 = -H$
1c: $R^1 = -CH_2COOCH_2CH_3$, $R^2 = -C(CH_3)_3$
1d: $R^1 = -(CH_2)_{15}CH_3$, $R^2 = -H$



2 (cone conformer)



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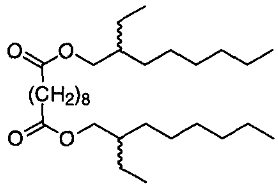
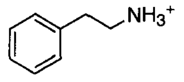
Calix[6]arene hexaesters **1a**,[2] **1b**,[3a] **1c**,[3a,b] and homooxacalix[3]arene triether (**2**, cone conformer)[4] were synthesized by employing the literature methods. Calix[6]arene hexaether (**1d**) was synthesized by an ordinary method.[5] PVC matrix liquid membranes incorporated with hosts **1a**, **1b**, **1d**, **2** and **3** (membranes 1a, 1b, 1d, 2 and 3, respectively) were prepared with bis(2-ethylhexyl) sebacate ["dioctyl sebacate" (DOS)] as a membrane solvent and PVC as a polymer matrix.[6] Sodium tetrakis[3,5-bis(1,1,1,3,3,3-hexafluoro-2-methoxy-2-propyl)-phenyl]borate (HFPB) was added to all membranes as a lipophilic anionic site [host:HFPB = 1:0.30 (molar ratio)] to obtain stable and reproducible membrane potentials. The electrode cell for the potential measurements was as follows: Ag | AgCl | 3 M KCl ⋮ 0.1 M $CH_3COOLi–CH_3COOH$ (pH 5.0) ⋮ sample solution | membrane | 10^{-2} M KCl | AgCl | Ag. Potentiometric responses to simple amines with various nonpolar structures (**4**~**8**), dopamine (**9**) and other catecholamines (**10**, **11**), and inorganic cations (K^+, Na^+) were measured at pH 5.0. At this pH, all of the amine guests are almost completely in their protonated, monocationic forms.

Potential vs concentration curves (potentiometric response curves) for membranes 1a and 2 are shown in Figure 1. Membrane potentials (electromotive forces, EMF) increased with increasing concentration of guest, showing cationic responses due to complexation between monocationic guests and neutral hosts at the membrane boundary region. Relative magnitudes of membrane potential changes are listed in Table 1 as potentiometric selectivity coefficients ($K^{pot}_{A,B}$),[7] except for membrane 1d which showed only weak responses. As an index for the lipophilicity of the amine guests, partition coefficients for 1-octanol/water system (log P_{oct}) are also listed.[8]


DOS (membrane solvent)

$CH_3(CH_2)_7NH_3^+$

4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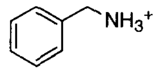
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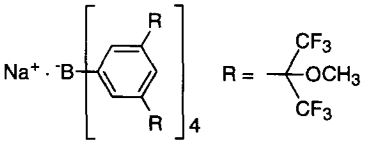
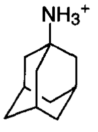

HFPB (lipophilic anionic site)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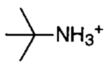
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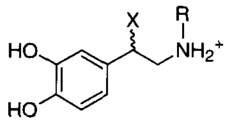
9: X = R = H (dopamine)
10: X = OH, R = H (noradrenaline)
11: X = OH, R = CH_3 (adrenaline)

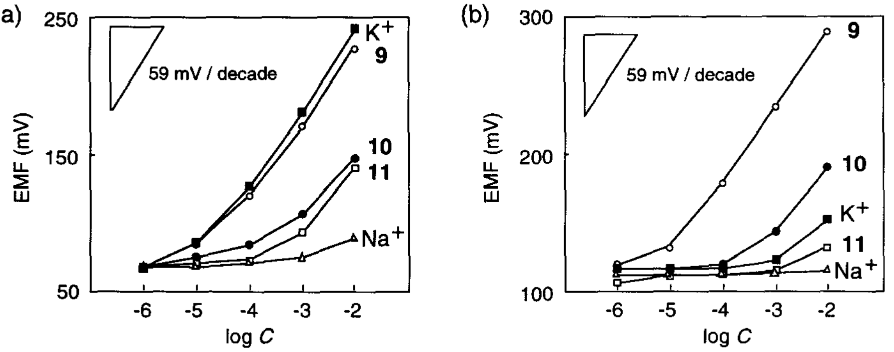

Figure 1. Potential vs concentration curves for dopamine (**9**), noradrenaline (**10**), adrenaline (**11**), and inorganic ions (K^+, Na^+), obtained at pH 5.0 by PVC matrix liquid membranes incorporated with (a) calix[6]arene hexaester (**1a**) and (b) homooxacalix[3]arene triether (**2**).

Table 1. Selectivities of Guest-induced Changes in Membrane Potential ($K^{pot}_{A,B}$) at pH 5.0 for PVC Matrix Liquid Membranes Incorporated with Hosts (**1a**, **1b**, **2**, **3**) and Blank Membrane without Host [a]

		membrane [b]				
guest [R-NH_3^+]	log P_{oct} [c]	1a	1b	2	3	blank
Group 1						
4 [R = $CH_3(CH_2)_7$-]	2.93	2.58	3.52	2.01	19.0	1.68
5 [R = $Ph(CH_2)_2$-]	1.58	1	1	1	1	1
6 [R = $PhCH_2$-]	1.09	0.15	0.15	0.45	0.90	0.78
7 [R = 1-adamantyl]	1.77	< 0.01	< 0.01	1.85	14.8	1.20
8 [R = $(CH_3)_3C$-]	0.72	< 0.01	< 0.01	0.31	0.40	0.56
Group 2						
9 [dopamine]	0.82	1	1	1	1	1
10 [noradrenaline]	0.82	0.10	0.096	< 0.01	0.89	0.62
11 [adrenaline]	0.59	0.089	0.090	< 0.01	0.65	0.65
K^+		5.24	7.02	0.0065		1.22
Na^+		0.031	0.060	0.00060		0.70

[a] Potentiometric selectivities are given by selectivity coefficients ($K^{pot}_{A,B}$), determined for each group of guests at pH 5.0 (0.1 M CH_3CO_2Li-CH_3COOH buffer) with **5** or **9** as a standard.[7] [b] For the compositions of the membranes, see References and Notes.[6] [c] P_{oct}: partition coefficient for 1-octanol/water system.[8]

The following points are evident from Table 1.

(1) The potentiometric selectivities of calix[6]arene esters for both groups of guests were essentially unaffected by the length of the alkyl chains [**1a** (C_{10}) vs **1b** (C_2)]. Both hosts showed selectivities to non-branched amines (**4**, **5**, **9**) based on the availability of stable inclusion complexes.[2] These selectivities contrast the selectivities governed by the lipophilicity factor of guests (log P_{oct}), as observed for membrane 3 incorporated with dibenzo-18-crown-6 or blank membrane without added host (containing only HFPB as a sensory element).

(2) The calix[6]arene esters (**1a**, **1b**) showed a good selectivity to dopamine (**9**) against noradrenaline (**10**) and adrenaline (**11**) but not against K^+ ion ($K^{pot}_{dopamine,\,K} = 5\sim7$). Such a poor dopamine/$K^+$ selectivity would cause severe interference by K^+ ion in potentiometric measurements of dopamine in biological samples.

(3) In contrast to the calix[6]arene esters (**1a**, **1b**), the homooxacalix[3]arene host (**2**), which is also capable of forming host-guest complexes with protonated primary amines by the involvement of tripodal hydrogen bonds,[4] did not show potentiometric discrimination of the structures of nonpolar moieties of group 1 guests (**4**~**8**), but showed an improved selectivity to dopamine against other catecholamines (**9** vs **10**, **11**; $K^{pot} < 0.01$). Furthermore, the dopamine/K^+ selectivity was dramatically improved, as clearly seen in Figure 1b and indicated by the $K^{pot}_{A,B}$ values in Table 1 ($K^{pot}_{dopamine,\,K} = 6.5 \times 10^{-3}$, $K^{pot}_{dopamine,\,Na} = 6.0 \times 10^{-4}$).

Shinkai *et al.*[4] have reported preferential extraction of 1-butylammonium ion over K^+ and Na^+ ions by **2** and the corresponding triethyl ether host in water-dichloromethane system, and on the basis of ^1H-NMR study, showed that uncomplexed homooxacalix[3]arene triethers adopt a flattened conformation. Based on these observations, it can be speculated that the flattened conformation of host **2** reduces the size of the binding site composed of three phenolic oxygens, disfavoring the formation of *inclusion* complexes with K^+ ion while retaining the ability to form a *nesting* complex with unhindered protonated amines such as dopamine.

The 150-fold preference to dopamine over K^+ ion in the membrane potential change attained by the homooxacalix[3]arene host (**2**) is quite attractive because it illustrates a promising approach to more sensitive and selective hosts that enable the monitoring of dopamine existing in low concentraions (for example, 10^{-2}~10^{-4} mM in normal or diseased human brain samples) in the presence of high concentrations of K^+ and Na^+ ions (~4 mM and ~140 mM, respectively). Efforts for elucidation of the details of dopamine selectivity are now in progress.

References and Notes

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(2) Odashima, K.; Yagi, K.; Tohda, K.; Umezawa, Y. *Anal. Chem.* **1993**, *65*, 1074-1083.

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(5) Calix[6]arene hexaether (**1d**) was prepared by exhaustive *O*-alkylation of 37,38,39,40,41,42-hexahydroxycalix[6]arene with 1-bromohexadecane and K_2CO_3 in 2-butanone (reflux, 70 h; purified yield 44%). White crystals: mp 184-185 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (t, J = 6.5 Hz, 18 H, CH_3), 1.24 [br s, 180 H, $O(CH_2)_{15}CH_3$], 3.89 (br s, 12 H, $ArCH_2$), 6.75 [br, 6 H, Ar*H*(para)], 6.95 [br, 12 H, Ar*H*(meta)]. IR (KBr) ν_{max} 2920, 2851, 1589, 1196, 1080 cm^{-1}. FDMS *m/z* 1981 (M^+ + 1). Anal. Calcd for $C_{138}H_{228}O_6$: C, 83.64; H, 11.52. Found: C, 83.39; H, 11.40.

(6) The PVC matrix liquid membranes were prepared by the procedure described previously (Tohda, K.; Tange, M.; Odashima, K.; Umezawa, Y.; Furuta, H.; Sessler, J. L. *Anal. Chem.* **1992**, *64*, 960-964). The membrane compositions [host : DOS (membrane solvent) : PVC (polymer matrix) : HFPB (anionic site), w/w] were as follows: Membrane **1a**, 5:68:27:1.5. Membrane **1b**, 3:69:28:1.4. Membrane **1d**, 6:67:27:1.4. Membrane **2**, 3:68:29:2. Membrane **3**, 1:66:32:2. Membrane **1c** could not be prepared because of insufficient solubility of **1c** in DOS. A membrane without added host (blank membrane; DOS:PVC:HFPB = 2:66:32 w/w) was also prepared in a similar manner. PVC from Wako Pure Chemical (Osaka, Japan) (average degree of polymerization ≈ 1100) was used.

(7) Potentiometric selectivity coefficients ($K^{pot}_{A,B}$) for group 1 (**4**~**8**) and group 2 (**9**~**11**, K^+, Na^+) guests were determined at room temperature (*ca.* 25 °C) by the matched potential method in mixed solutions. [See: (a) Gadzekpo, V. P. Y.; Christian, G. D. *Anal. Chim. Acta* **1984**, *164*, 279-282. (b) Umezawa, Y.; Umezawa, K.; Sato, H. *Pure Appl. Chem.* **1995**, *67*, 507-518]. In this method, the selectivity coefficient is defined as the ratio of the concentrations of the primary and interfering ions which give the same potential change under the same background condition. In the present study, a 1.00×10^{-4} concentration of **5** or **9** was used as a background condition for groups 1 and 2, respectively. The potentials were recorded when the EMF increase became smaller than 0.5 mV min^{-1}.

(8) The partition coefficients for 1-octanol/water system (log P_{oct}) for the organic amine guests (**4**~**11**) at room temperature (*ca.* 25 °C) were calculated from the R_f values of reversed phase thin layer chromatography [Wattman-KC18F silica gel plate (5 × 20 cm)] according to the method reported by Simon *et al.* The guests and standard materials (*p*-chlorophenol, phenol, *p*-benzoquinone, *p*-aminophenol) were developed with ethanol / 0.1 M CH_3COOLi–CH_3COOH (pH 5.0) (20:80 v/v). The R_f values of each guest were estimated from those of the standard materials described in the literature. See: Dinten, O.; Spichiger, U. E.; Chaniotakis, N.; Gehrig, P.; Rusterholz, B.; Morf, W. E.; Simon, W. *Anal. Chem.* **1991**, *63*, 596-603.