Journal of Inclusion Phenomena and Macrocyclic Chemistry **34:** 259–266, 1999.

Study of the Complexation of Octakis(diethoxyphosphoryloxy)-tetramethylcalix[4]resorcinarene with Benzene Derivatives by the RP HPLC Method

O. I. KALCHENKO, A. V. SOLOVYOV and V. I. KALCHENKO*
Institute of Organic Chemistry, National Academy of Sciences of Ukraine, Murmanskaya, 5, 253660, Kiev-94, Ukraine.

J. LIPKOWSKI
Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka, 44/52, Warsaw, Poland.

(Received: 25 May 1998; in final form: 14 October 1998)

Abstract. Stability constants of the host–guest complexes of octakis(diethoxyphosphoryloxy) tetramethylcalix[4]resorcinarene (RA) with different benzene derivatives (alkyl benzenes, halogenated benzenes, substituted aldehydes, phenols and benzoic acids) were determined by the reversed phase high-performance liquid chromatography (RP HPLC) method (Separon SGX C 18, UV detector at 254 nm and acetonitrile-water, 86 : 14, v/v, as mobile phase) from the relationship between the aromatic guest capacity factors and the RA host concentration in the mobile phase. The constants are within the range 17–596 M^{-1}, dependent on the size, nature, position and quantity of the substituents in the benzene ring of the guest molecules.

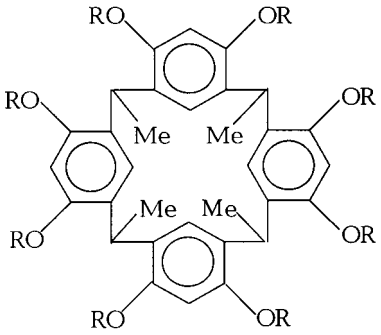
Key words: reversed phase high-performance liquid chromatography, calix[4]resorcinarene, host–guest complexes, stability constants.

1. Introduction

Calix[4]resorcinarenes composed of four resorcine units linked via alkylidene groups are intensively investigated as host molecules able to recognize guests of different nature due to their cavity shaped architecture [1, 2]. The complexing properties of the calixresorcinarenes have been examined in solution [3, 4], the crystalline state [5–8], the gaseous phase [9] and two dimensional solids (Langmuir Blodgett (LB) films) [4, 10–12]. The binding of metal cations, anions, organic and inorganic molecules has been demonstrated. The calixresorcinarene derivatives have been used for the design of sensor devices which are able to detect hydrogen chloride, ammonia [12] and aromatic molecules [10, 11, 13, 14] in air, and to recognize sugars in aqueous solution [4].

* Author for correspondence.

In order to increase the binding properties of calixresorcinarenes their phosphorus containing derivatives were synthesized and investigated [15–19]. One of the compounds synthesized, i.e., octakis(diphenoxyphosphoryloxy) tetramethylcalix[4]resorcinarene (RA) was used for the separation of benzene derivatives by the extractive crystallization method [7].



R = $(PhO)_2P(O)$

In this work we investigated host–guest complexation of the easily available RA with different benzene derivatives in acetonitrile-water solution. The reversed phase high-performance liquid chromatography method was used for this aim. The stability constants of the complexes were determined under these conditions. The data obtained could be useful in the design of calixarene based sensors.

2. Experimental

2.1. MATERIALS

All samples of benzene derivatives were of the best quality available and were purchased from various suppliers. RA was prepared by reaction of tetramethylcalix[4]resorcinareneoctol with diphenylchlorophosphate and triethylamine in acetonitrile solution [15]. RA and the starting tetramethylcalix[4]resorcinareneoctol are now available from Acros Organic.

2.2. RP HPLC ANALYSIS

The mobile phase, i.e., acetonitrile-water eluent containing RA at concentrations of 1.6×10^{-3}; 3×10^{-3} and 4.5×10^{-3} M was prepared by dissolving the RA in acetonitrile–water (86 : 14, v/v) solution at the ambient temperature. Analysis for each RA concentration was performed three times. The aromatic guest solutions for injections with concentration of each solute(s) of 0.5×10^{-4} M were prepared using a solvent identical with the mobile phase. The amount of the sample injected

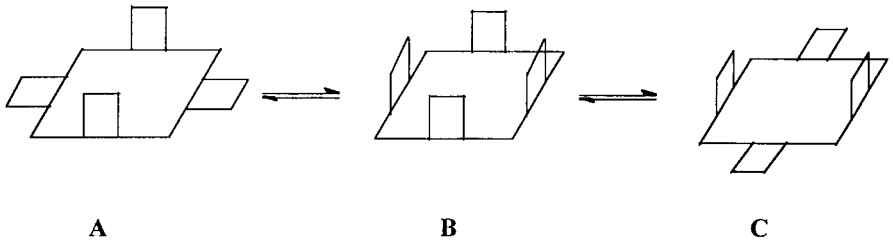


Figure 1. Schematic representation of the pseudorotation process (**A**, **C** – *boat*, **B** – *crown* conformation).

was 20 μL. Each of the samples was analyzed five times. The dead time, t_0, was determined by using $NaNO_2$. The retention times of the solutes were determined after 7 h elution when the column was saturated with RA. The RA capacity factor, k', was 8.65.

2.3. MEASUREMENTS

The liquid chromatographic system consisted of a UV-visible detector, high-pressure pump HPP 4001 (Czech Republic) connected to a Rheodyne Model sample 7120 injector (20 μL, Rheodyne Inc., Berceley, CA). The column (150 × 3.3 mm I.D.) was packed with Separon SGX C 18 (LaChema Czech Republic).

3. Results and Discussion

The stereochemistry of octaphosphorylated calix[4]resorcinarenes was first investigated in detail by the ^{1}H NMR method [15, 20]. In accordance with the data the RA molecule exists in the so called *boat* conformation in which two opposite benzene rings are coplanar with the main macrocyclic plane formed by carbon atoms of the methine bridges and the two others are perpendicular to the plane (C_{2v} symmetry). A similar *boat* conformation, investigated in detail by X-Ray crystallography, is observed in octakis(tosyloxy)- and tetrakis(dipropoxyphosphoryloxy)tetramethylcalix[4]resorcinarenes [7, 21].

The RA *boat* conformation is stereochemically flexible. The rapid pseudorotation process (Figure 1) consisting in site exchange of the benzene rings with the coplanar and perpendicular orientation is observed in solution at room temperature. The *crown* conformation is intermediate in this transition. The activation energy of the process is strongly dependent on the solvent ($(CD_3)_2CO > CDCl_3 > CCl_4$) [20].

In view of the above consideration it might be suggested that complexation of guest species by the RA host molecule is associated with the conformational flexibility of the macrocyclic skeleton formed by the four benzene rings.

Host–guest complexation of calixarenes with organic guest molecules in solutions are usually investigated by the NMR method. Recently the reversed phase high-performance liquid chromatography method was used for the investigation

of the complexing properties of calixarenes [21, 23]. It was shown that addition of calixarenes or calixresorcinarenes to the mobile phase leads to the reduction of retention times of aromatic molecules investigated as solutes. The reduction of the retention times is explained by formation of host–guest inclusion complexes of the macrocyclic hosts with aromatic molecules in solution. This complexation weakens the interaction of the solutes with the stationary phase. The stability constants of the complexes were determined from the relationship between the aromatic guest capacity factors and the macrocyclic host concentration in the mobile phase.

This method was used for the stability constant determination of the complexes of RA with a number of benzene derivatives (Table I) in acetonitrile–water (86 : 14, v/v) solution. The investigated guest molecules bear at the benzene ring substituents of different size and nature: chlorine and bromine atoms, methyl, trichloromethyl, *t*-butyl, nitro, amino, hydroxyl, formyl and carboxyl groups. These substituents determine the capacity factors, k'_0, of the solutes, as well as the efficiency of the host–guest complexation. To calculate the stability constants of the host–guest complexes Equation (1) [21, 23, 24] was used:

$$1/k' = 1/k'_0 + [\mathrm{RA}]/K_\mathrm{A} \times k'_0 \tag{1}$$

where k'_0 and k' are the capacity factors in the absence and presence of RA in the mobile phase; [RA] is the concentration of RA in the mobile phase and K_A is the stability constant of the complex.

The main conditions of the correct utilization of Equation (1) are as follows: Firstly, the linear dependence of k' vs. RA concentration in the range from 1.6×10^{-3} to 4.5×10^{-3} M (Table I) confirms formation of the host–guest complexes in a 1 : 1 composition; secondly, the sorption of RA as well as RA-solute complexes on the stationary phase may be neglected. These assumptions are confirmed by the following data. The plate number of the column as well as the asymmetry coefficient of the RA chromatographic peak (1.15) do not depend on the RA concentration in the mobile phase. For this reason [RA] must be similar to the RA concentration indicated in Table I.

The stability constants K_A of the inclusion complexes formed by RA and aromatic guests calculated from Equation (1) are presented in Table I. The stability constants lie in the region from 17 M^{-1} (*tert*-butylbenzene) to 598 M^{-1} (*p*-bromotoluene) dependent on the size, nature, quantity and position of the substituents (fluorine, chlorine, bromine atoms, methyl, methoxyl, trifluoro (trichloro)methyl, *tert*-butyl, hydroxyl, nitro, amino, formyl and carboxyl groups) at the benzene rings of the guest molecules. The substituents determine the manner and efficiency of the host–guest binding as a result of the different physical interactions: coulombic dipole-dipole forces, hydrogen bonds, solvatophobic interactions etc. Sometimes, the substituents influence the bonding of the guests molecules in the opposite directions. For example, amino, hydroxyl and carboxyl groups increase the complexation efficiency due to the hydrogen bond formation with oxygen atoms of the RA P=O groups. On the other hand, these substituents

Table I. Capacity factors of the guests k'_0, k', $\Delta k' = k' - k'_0$ and the host–guest stability constants K_A

		k'_0	$k'(\Delta k')$			K_A, M^{-1}
		No add.	1.6×10^{-3} M	3×10^{-3} M	4.5×10^{-3} M	(RSD, %)
Alkylated benzenes:						
1	*tert*-butyl-Benzene	1.21	1.18(0.03)	1.15(0.06)	1.11(0.10)	17(11.48)
2	Toluene	1.42	1.34(0.08)	1.28(0.14)	1.25(0.17)	59(11.14)
3	Benzene	1.08	0.99(0.09)	0.90(0.18)	0.88(0.20)	61(10.19)
4	*m*-Xylene	2.18	1.67(0.51)	1.56(0.62)	1.54(0.64)	131(5.0)
5	Trichloromethylbenzene	1.28	1.02(0.26)	0.90(0.38)	0.85(0.43)	157(9.46)
6	Trifluoromethylbenzene	1.54	0.93(0.61)	0.72(0.82)	0.48(1.06)	428 (5.35)
	Halogenated benzenes:					
7	*o*-Dichlorobenzene	1.43	1.34(0.09)	1.25(0.18)	1.23(0.20)	48(12.51)
8	*p*-Dichlorobenzene	1.54	1.44(0.10)	1.32(0.22)	1.25 (0.29)	52(8.16)
9	Chlorobenzene	0.78	0.70(0.08)	0.64(0.14)	0.60(0.18)	71(4.34)
10	*o*-Bromotoluene	2.46	1.35(0.11)	1.0(1.46)	0.93(1.56)	538(6.47)
11	*p*-Bromotoluene	1.87	0.95(0.92)	0.69(1.18)	0.53(1.34)	596 (3.32)
Aldehydes:						
12	Salicyl aldehyde	0.67	0.63(0.04)	0.60 (0.07)	0.57 (0.10)	40(3.88)
13	Veratraldehyde	1.01	0.89(0.12)	0.82(0.19)	0.76(0.25)	78(6.52)
14	Benzaldehyde	0.73	0.65(0.08)	0.58(0.15)	0.53(0.20)	82(5.07)
15	Anisaldehyde	1.0	0.87(0.13)	0.80(0.20)	0.71(0.29)	89(6.10)
Substituted phenols:						
16	Veratrole	0.73	0.65(0.18)	0.58(0.15)	0.54(0.19)	80(5.37)
17	*p*-Chlorophenol	1.0	0.87(0.13)	0.80(0.20)	0.70(0.30)	91(4.68)
18	*p*-Cresol	1.0	0.87(0.13)	0.77(0.23)	0.70(0.30)	97(3.31)
19	*p*-Fluorophenol	0.92	0.87(0.05)	0.68(0.24)	0.65(0.27)	100(13.87)
20	1,2,3-Trihydroxybenzene	0.60	0.50(0.10)	0.46(0.14)	0.39(0.21)	114(10.97)
21	*p*-Bromophenol	1.0	0.82(0.18)	0.76(0.24)	0.68(0.32)	116(15.91)
22	Phenol	0.97	0.81(0.16)	0.73(0.240	0.60(0.37)	123(11.44)
23	Guaicol	1.95	1.45(0.50)	1.23(0.72)	1.0(0.95)	209(6.01)
24	*p*-Aminophenol	0.60	0.40(0.20)	0.31(0.29)	0.25(0.35)	311(0.16)
Carboxylic acids:						
25	*p*-Toluic	1.13	1.11(0.02)	1.09(0.04)	1.06(0.05)	29 (3.91)
26	*m*-Toluic	1.11	1.0(0.11)	0.94(0.17)	0.86(0.25)	64(7.95)
27	β-Phenyl Acrylic	0.92	0.82(0.10)	0.74 (0.18)	0.68(0.24)	77(13.86)
28	*o*-Phthalic	1.09	0.94(0.15)	0.86(0.23)	0.73(0.36)	97(11.33)
29	*p*-Nitrobenzoic	0.73	0.63(0.10)	0.55(0.18)	0.52(0.21)	100(10.21)
30	*p*-Cumaric	0.95	0.80(0.15)	0.69 (0.26)	0.60(0.35)	128(2.38)
31	*o*-Nitrobenzoic	0.61	0.50(0.11)	0.44(0.17)	0.38(0.23)	133(3.53)
32	*o*-Chlorobenzoic	0.53	0.43(0.10)	0.38(0.15)	0.32(0.21)	141(6.23)
33	*p*-Chlorobenzoic	0.65	0.52(0.13)	0.46(0.19)	0.40(0.25)	144 (7.27)
34	*p*-Hydroxybenzoic	0.60	0.49(0.11)	0.41(0.19)	0.36(0.24)	146(5.21)
35	3,4,5-Trihydroxybenzoic	0.71	0.57(0.14)	0.50(0.21)	0.42(0.29)	149(5.17)
36	*o*-Aminobenzoic	0.10	0.08(0.02)	0.07(0.03)	0.06(0.04)	149 (4.40)
37	*m*-Hydroxybenzoic	0.22	0.18(0.04)	0.16(0.06)	0.12(0.10)	149(21.30)
38	Benzoic	0.83	0.67(0.16)	0.57(0.26)	0.50(0.33)	150(1.50)
39	*m*-Aminobenzoic	1.0	0.80(0.20)	0.66(0.34)	0.58(0.42)	163(5.48)
40	*p*-Aminobenzoic	0.60	0.48(0.12)	0.42(0.18)	0.32(0.28)	164(16.52)
41	*o*-Fluorobenzoic	0.20	0.16(0.04)	0.12(0.08)	0.10(0.10)	209(10.57)
42	*m*-Nitrobenzoic	0.72	0.51(0.21)	0.41(0.10)	0.36(0.36)	244(7.65)
43	*p*-Phthalic	0.83	0.50(0.33)	0.40(0.43)	0.39(0.44)	389(9.25)

lead to an increase of the guests hydrophilicity and a decrease of their solvatophobic interactions with RA molecules in acetonitrile–water solution. Solvatophobic interactions increase the influence of the lipophilic alkyl groups. But, bulky alkyl groups hinder the complexation process sterically. The discussion of the calculated constants reflects the complicated character of the substituent groups on the host–guest interactions.

The stability constants of benzene, toluene, chlorobenzene, dichlorobenzene (*o*- and *p*-isomers), veratrole, as well as benzaldehyde and its derivatives (excluding salicyl aldehyde) have similar values from 59 to 89 M^{-1}. At the same time the introduction in the benzene molecule (K_A = 61 M^{-1}) of the bulky *tert*-butyl group restricts the inclusion of the guest molecule in the RA cavity and leads to a three fold decrease of K_A (K_A = 17 M^{-1}).

Phenols interact with RA more effectively (K_A = 91–311 M^{-1}) due to the additional hydrogen bond formation with phosphoryl groups. The largest K_A is observed for *p*-aminophenol (311 M^{-1}) due to hydrogen bond formation, N—H···O=P, between the amino and P=O group. At the same time the OH group in the salicyl aldehyde reduces the host–guest interaction by the intramolecular hydrogen bond OH···O=C and the constant stability is only 40 M^{-1}. The replacement of the OH group in the guaicol molecule (K_A = 209 M^{-1}) by a OCH_3 group leads to a decrease in K_A of 2.5 times (K_A for the veratrole is only 80 M^{-1}). This may be explained by the hydrogen bond formation between the OH groups of the guaicol molecule and the P=O groups of the RA molecule.

Stronger complexes (K_A = 97–398 M^{-1}) were observed for carboxylic acids (excluding *p*- and *m*-toluic acids with K_A = 29 and 64 M^{-1}). The largest K_A is observed for *p*-phthalic acid (389 M^{-1}), where two COOH groups form hydrogen bonds with the RA molecule. The K_A of the *o*-phthalic acid is only 97 M^{-1} due to the formation of the hydrogen bonds between neighbouring COOH groups.

The highest stability constants among the investigated benzene derivatives are given by *o*-bromotoluene (K_A = 538 M^{-1}) and *p*-bromotoluene (K_A =596 M^{-1}). A similar increase of the stability constants influenced by the insertion of bromine atoms into the aromatic guest molecule was also observed for the complexes of *alpha*-cyclodextrin with butyrophenone derivatives [24]. For example spectrophotometric investigation of the complexation in aqueous solution showed that the replacement of Cl in the *p*-chlorobutyrophenone guest molecule (K_A = 72 M^{-1}) by Br leads to a tenfold increase in the K_A (K_A = 760 M^{-1}).

The stability constants presented in Table I are similar to the K_A values of *alpha*-cyclodextrin complexes of the aromatic molecules in methanol-water solution (50 : 50) determined by the RP HPLC method [25]. So, K_A values of the *alpha*-cyclodextrin complexes were 4 M^{-1} (*p*-nitroaniline), 84 M^{-1} (*p*-cresol) and 129 M^{-1} (*p*-nitrophenol). The K_A values of the complexes in water where the solvatophobic (hydrophobic) interactions play the main role were larger (K_A = 2045, 408, and 1148 M^{-1} respectively).

The presented data testify that the RA complexing ability in respect to the aromatic guest molecules is similar to the ability of *alpha*-cyclodextrin, which up to now is considered as one of the classical host molecules in supramolecular chemistry.

4. Conclusion

The easily available octakis(diphenoxyphosphoryloxy)calix[4]resorcinarene forms host–guest complexes with benzene and its different alkyl, halogen, nitro, amino, hydroxyl, formyl, carboxyl derivatives in acetonitrile-water solution. The association constants of the complexes range from 17 to 596 M^{-1} dependent on the size, nature, position and quantity of substituents on the benzene ring of the guest molecule. The RA complexing ability is similar to the ability of *alpha*-cyclodextrin under these conditions.

Acknowledgements

This work is performed in accordance with the scientific cooperation between the Institute of Physical Chemistry, Polish Academy of Sciences and the Institute of Organic Chemistry, National Academy of Sciences of Ukraine. The authors of the Kiev group thank INTAS for partial financial support of the work through grant 95–129.

References

1. P. Timmerman, W. Verboom, and D. N. Reinhoudt: *Tetrahedron* **52**, 2663 (1996).
2. V. Böhmer: *Angew. Chem. Int. Ed. Engl.* **34**, 713 (1995).
3. K. Kobayashi, Y. Asakywa, Y. Kato, and Y. Aoyama: *J Am. Chem. Soc.* **114**, 10307 (1992).
4. K. Kurihara, K. Ohto, Y. Tanaka. Y. Aoyama, and T. Kunitake: *J. Am. Chem. Soc.* **113**, 444 (1991)
5. D. J. Cram: *Nature* **356**, 29 (1992).
6. D. J. Cram, L. M. Tunstad, and C. B. Knobler: *J. Org. Chem.* **57**, 528 (1992).
7. V. I. Kalchenko, A. V. Solovyov, M. R. Gladun, A. N. Shivanyuk, L. I. Atamas, V. V. Pirozhenko, L. N. Markovsky, J. Lipkowski, and Yu. A. Simonov: *Supramol. Chem.* **8**, 269 (1997).
8. W. Xu, J. P. Rourke, J. J. Vittal, and R. J. Puddephat: *J. Am. Chem. Soc.* **115**, 6456 (1993).
9. M. Vincenti, E. Dalcanale, P. Soncini, and G. Guldimetti: *J. Am. Chem. Soc.* **112**, 445 (1990).
10. A. V. Nabok, N. V. Lavrik, Z. I. Kazantseva, B. A. Nesterenko, L. N. Markovsky, V. I. Kalchenko, and A. N. Shivanyuk: *Thin Solid Films* **259**, 244 (1995).
11. A. V. Nabok, A. K. Hassan, A. K. Ray, O. Omar, and V. I. Kalchenko: *Sensors and Actuators B* **45**, 115 (1997).
12. N. V. Lavrik, D. DeRossi, Z. I. Kazantseva, A. V. Nabok, B. A. Nesterenko, S. A. Piletsky, V. I. Kalchenko, A. N. Shivanyuk, and L. N. Markovsky: *Nanotechnology* **7**, 315 (1996).
13. E. Dalcanale and J. Hartman: *Sensors and Actuators B* **24**, 39 (1995).
14. P. Nelli, E. Dalcanale, G. Faglia, G. Sverveglien, and P. Soncini: *Sensors and Actuators B* **13**, 302 (1993).

15. V. I. Kalchenko, D. M. Rudkevich, A. N. Shivanyuk, I. F. Tsimbal, V. V. Pirozhenko, and L. N. Markovsky: *Russian J. Gen. Chem.* **64**, 663 (1994).
16. T. Lippman, E. Dalcanale, and G. Mann: *Tetrahedron Lett.* **35**, 1688 (1994).
17. P. Delangle and J.-P. Dutasta: *Tetrahedron Lett.* **36**, 9325 (1995).
18. V. I. Maslennikova, E. V. Panina, A. R. Bekker, L. K. Vasyanina, and E. E. Nifantyev: *Phosphorus, Sulfur and Silicon* **113**, 219 (1996).
19. W. Xu, J. J. Vital, and R. J. Puddephatt: *J. Am. Chem. Soc.* **117**, 8362 (1995).
20. A. N. Shivanyuk, V. V. Pirozhenko, V. I. Kalchenko, and L. N. Markovsky: *J. Chem. Res. (S)* 374 (1995).
21. J. Lipkowski, O. I. Kalchenko, J. Slowikowska, V. I. Kalchenko, O. V. Lukin, L. N. Markovsky, and R. Nowakowski: *J. Phys. Org. Chem.* **11**, 426 (1998).
22. O. I. Kalchenko, J. Lipkowski, R. Nowakowski, V. I. Kalchenko, M. A. Vysotsky, and L. N. Markovsky: *J. Chromatogr. Sci.* **35**, 49 (1997).
23. O. I. Kalchenko, J. Lipkowski, V. I. Kalchenko, M. A. Vysotsky, and L. N. Markovsky: *J. Chromatogr. Sci.* **36**, 269 (1998).
24. K. Fujimura, T. Ueda, M. Kitagawa, H. Takayanagi, and T. Ando: *Anal. Chem.* **58**, 2668 (1986).
25. D. M. Davies, M. E. Deary, and D. J. Wealleans: *J. Chem. Soc., Perkin Trans. 2*, 193 (1998)