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Host–guest interactions of calix[4]resorcinarenes with benzene derivatives in conditions of reversed-phase high-performance liquid chromatography. Determination of stability constants

J. Lipkowski,[1]* O. I. Kalchenko,[1]† J. Slowikowska,[1] V. I. Kalchenko,[2] O. V. Lukin,[2] L. N. Markovsky[2] and R. Nowakowski[1]

[1]Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland
[2]Institute of Organic Chemistry, National Academy of Sciences of Ukraine, Murmanskaja 5, 253660, Kiev-94, Ukraine

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ABSTRACT: Reversed-phase high-performance liquid chromatography [LiChrosorb RP-18,UV detection at 254 nm and acetonitrile-water (86:14,v/v) as mobile phase] was applied to studies of the host–guest complexation of tetraalkylcalix[4]resorcinareneoctols and their upper rim phosphoryl, sulfonyl and dialkylaminomethyl derivatives with some aromatic guests in the mobile phase. It was shown that the formation of the inclusion complexes results in changes in the retention of aromatic guests and improves their separation. Stability constants of the complexes were calculated from the dependences of the l/k' values of the aromatic guest on the concentration of the calix[4]resorcinarene in the mobile phase. The molecular structure of 4,6,16,18-tetrahydroxy-10,12,22,24-tetrakis(dipropoxyphosphoryloxy)-2,8,14,20-tetramethylcalix[4]resorcinarene (12) was determined. Crystal data for 12 are $P2_1/n$, $a = 16.708(9)$ Å, $b = 18.683(6)$ Å, $c = 20.243(5)$ Å, $\beta = 95.75(3)°$, $V = 6287(4)$ Å^3 and $Z = 4$. Compound 12 exists in a boat conformation, in which two opposite unsubstituted resorcinol rings of the macrocyclic skeleton lie in the plane formed by four methine bridges and two diphosphorylated rings are perpendicular to the plane. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: calix[4]resorcinarenes; organophosphorus compounds; solvatophobic interactions; host–guest complexation; high-performance liquid chromatography; x-ray analysis

INTRODUCTION

Cavity-shaped calix[4]resorcinareneoctols (Scheme 1) (The terms metacyclophanes and resolcarenes are also used for these compounds), made-up of four resorcinol units linked *via* alkylidene groups, are promising compounds for the design of host molecules able to bind organic and inorganic species[1] (Scheme 1). In order to increase the binding properties, numerous calix[4]resorcinareneoctols derivatives functionalized on oxygen atoms and also on carbon atoms of benzene rings were synthesized[2] and their complexing properties were examined in solutions,[3–7] the crystalline state,[8–10] the gaseous phase[11] and Langmuir–Blodgett films.[12,13] Effective complexants able to bind selectively aromatic molecules,[10] alkylammonium compounds,[4] metal cations[14] and anions[15,16] and to separate enantiomers[16] were selected as result of these investigations. The compounds were used for the design of sensor devices which are able to detect aromatic molecules in air[13] and to recognize sugars in aqueous solutions.[12]

In this work we investigated host–guest interactions of tetraalkycalix[4]resorcinareneoctols (**1–5**) and some of their upper rim nitrogen, phosphorus and sulfur derivatives (**6–12**) with benzene derivatives under conditions of reversed-phase high-performance liquid chromatography (RP-HPLC). The stability constants of the host–guest inclusion complexes were determined. The molecular structure of tetrakis(dipropoxyphosphoryloxy)tetramethylcalix[4]resorcinarene was determined by x-ray diffraction analysis.

EXPERIMENTAL

Reagents. Acetonitrile, benzene, toluene, *p*-cresol, *p*-xylene and ethylbenzene were of analytical grade and were used without further purification. Calix[4]resorcinareneoctols **1–5**[17] and **6,**[18] **7,8** and **11,**[19] tetrakis(amino-

**Correspondence to:* J. Lipkowski, Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland.
Email: klatrat@alfa.ichf.edu.pl
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†On leave of absence from the Institute of Organic Chemistry, National Academy of Sciences of Ukraine, Kiev, Ukraine.

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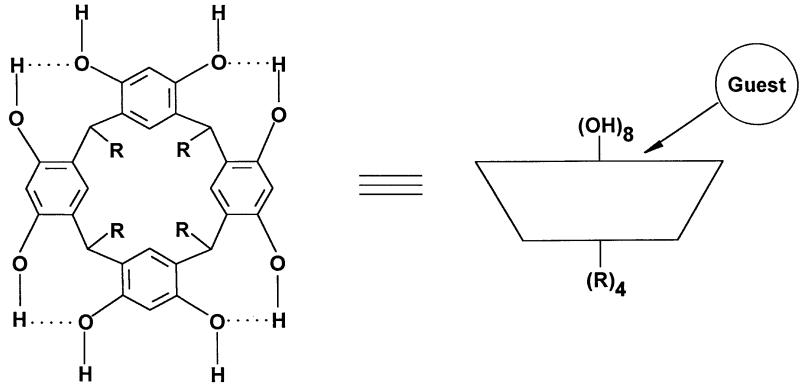
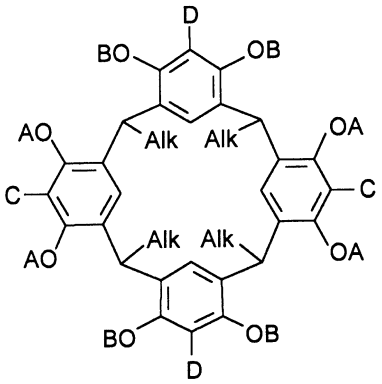


Scheme 1

methyl)calix[4]resorcinarene **9,**[10] the octatosylate **10**[20] and the tetraphosphate **12**[21] were synthesized by published methods. Tetramethyl- and tetrapenthylcalix-[4]resorcinareneoctols **1** and **3** are now comercially available from Acros Organics.

Crystal structure determination for 12. The crystal structure of tetrakis(dipropoxyphosphoryloxy)tetra-methylcalix[4]resorcinarene (**12**) was determined by x-ray diffraction analysis. A single colorless crystal of dimensions $0.25 \times 0.28 \times 0.35$ mm was sealed in a glass capillary with a small amount of mother liquor. Preliminary search and data collection were performed on a four-circle Enraf-Nonius CAD4 diffractometer with graphite-monochromated Cu K_α radiation at 200 K using the $\omega-2\theta$ scan technique. The unit cell parameters were derived by a least-squares procedure from 25 accurately centered reflections ($11.41° < \theta < 22.25°$). Three standard reflections were checked every 100 measurements and during data collection their intensities declined by 14%. Data were corrected for Lorentz and polarization effects, but not for absorption.



1-12

1	Alk = CH_3	A = B = C = D = H
2	Alk = C_3H_7	A = B = C = D = H
3	Alk = C_5H_7	A = B = C = D = H
4	Alk = C_7H_{15}	A = B = C = D = H
5	Alk = $C_{15}H_{31}$	A = B = C = D = H
6	Alk = CH_3	A = Ts, B = C = D = H
7	Alk = CH_3	A = p-$ClC_6H_4SO_2$, B = C = D = H
8	Alk = CH_3	A = Ts, B = C = H, D = $CH_2N(C_2H_5)_2$
9	Alk = CH_3	A = B = H, C = D = $CH_2N(C_6H_{13})_2$
10	Alk = C_3H_7	A = B = Ts, C = D = H
11	Alk = CH_3	A = $P(O)(OC_3H_7)_2$, B = C = H, D = $CH_2N(C_2H_5)_2$
12	Alk = CH_3	A = $P(O)(OC_3H_7)_2$, B = C = D = H

The structure was solved by direct methods[22] and refined by full-matrix least-squares calculations on $|F^2|$.[23] All non-hydrogen atoms were refined anisotropically. Carbon atoms in the propyl chains exhibiting high thermal parameters were refined as disordered. Some of the C—C bond lengths in the propyl chains were constrained to 1.54 Å. Hydrogen atoms were placed in idealized positions and their temperature factors were fixed at 1.2 (for CH, CH_2 and OH) and 1.5 (for CH_3) times the temperature factor of their parent C (or O) atom. Those hydrogen atoms attached to the calixarene backbone and methylene bridges were refined, while those in the propyl chains were subjected to a riding model refinement (C—H bonds fixed at 0.97 and 0.96 Å for CH_2 and CH_3, respectively). The final R index was equal to 0.077. Full experimental details and crystal data are given in Table 1.

HPLC analysis. The conditions for the RP-HPLC analysis of calix[4]resorcinarenes were as follows: a Type 333 HPLC unit (Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland) equipped with a 10^{-4} ml cell was used, the column (250×1 mm i.d.) was packed with LiChrosorb RP-18 (Merck, Darmstadt, Germany), the mobile phase was a solution containing acetonitrile–water (86: 14, v/v), the flow rate was 0.04 ml min^{-1} and UV detection was performed at 254 nm.

All measurements were performed at 21 °C. Mobile phases containing calix[4]resorcinareneoctols and their derivatives were obtained by dissolving the calixarene compounds in acetonitrile–water solution at concentrations of 1×10^{-3}, 2×10^{-3} and 3×10^{-3} M. Each of the three concentrations was analysed twice. Mobile phases with calix[4]resorcinarenes as additives were equili-

Table 1. Crystal data and structure refinement for compound **12**

Empirical formula	$C_{56}H_{84}O_{20}P_4$
Formula weight	1201.11
Temperature	200(2) K
Wavelength	1.54178 Å
Crystal system	Monoclinic
Space group	$P2_1/n$
Unit cell dimensions	a = 16.708(9) Å; b = 18.683(6) Å; c = 20.243(5) Å; β = 95.75(3)°;
Volume	6287(4) Å^3
Z	4
Density (calculated)	1.269 g cm^{-3}
Absorption coefficient	1.698 mm^{-1}
F(000)	2560
Crystal size	0.25 × 0.28 × 0.35 mm
Range of θ for data collection	3.23–77.83°
Index ranges	$-20 \leq h \leq 21$, $0 \leq k \leq 23$, $-25 \leq l \leq 0$
Reflections collected	13514
Independent reflections	13143 [R(int) = 0.0508]
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	11546/81/833
Goodness-of-fit on F^2	1.034
Final R indices [$I > 2\ \sigma\ (I)$]	$R1$ = 0.0774, $wR2$ = 0.2098
R indices (all data)	$R1$ = 0.1276, $wR2$ = 0.3028
Extinction coefficient	0.00079(10)
Largest diff. peak and hole	0.992 and −0.590 e/ Å^{-3}

brated for 7 h before analysis. The concentrations of benzene, ethylbenzene, *p*-xylene, *p*-cresol and toluene in the injected acetonitrile–water solutions were 1×10^{-4} M. Each of the samples was analysed five times. The standard deviation of K_A was determined from multiple analyses. The column dead volume was determined by injection of $NaNO_2$ solution.

RESULTS AND DISCUSSION

Macrocyclic compounds (cyclodextrins, calixarenes, crown ethers) are widely used in liquid chromatography as modifiers of stationary phases or as additives in mobile phases for improving the chromatographic characteristics of substances being separated, including enantiomeric mixtures.[24,25] Their behavior is based on the formation of host–guest inclusion complexes with analysed compounds (organic molecules or metal cations), that results in changes in the retention times of these compounds. In the series of macrocyclic host molecules α-, β- and γ-cyclodextrins are the most widely investigated. In their cavities formed by six, seven or eight α-1,4-D-glucopyranose units, with various architecture and sizes, guest molecules may be included and firmly retained owing to the different physical interactions (van der Waals forces, hydrogen bonds, hydrophobic interactions, etc.). calix[4]resorcinarenes are also able to participate in similar host–guest interactions.[26,27] Their molecular cavities may be formed by up-oriented benzene rings of a conformationally flexible macrocyclic skeleton. The architecture and volume of the macrocyclic cavity formed are determined by the nature of substituents at the upper rim of the macrocycle. Despite this cavity, the guest molecule may also be included in the cavity formed by four *cis*-oriented alkyl substituents at the lower rim of the macrocycle.[9]

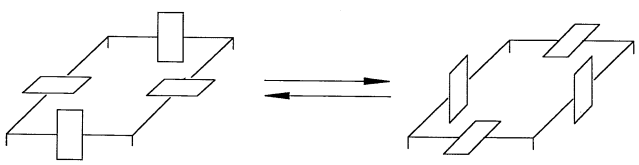


Figure 1. Boat–boat pseudo-rotation process

For this investigation we chose the calix[4]resorcinareneoctols **1–5** with all-*cis*-oriented alkyl substituents of different lengths at the lower rim of the macrocycle and their derivatives **6–12** functionalized at the upper rim of the macrocycle with phosphoryl, arylsulfonyl and *N*,*N*-dialkylaminomethyl groups (Scheme 2).

In accordance with literature data[28–30], all-*cis*-tetraalkylcalix[4]resorcinareneoctols **1–5** in solutions and in the solid state exist in a crown conformation with all resorcinol rings up-oriented (C_{4v} symmetry) stabilized in this position by intramolecular hydrogen bonds at the upper rim of the macrocycle. The crown conformation is probably realized also in tetrakis(diethylaminomethyl)-calix[4]resorcinareneoctol (**9**)[19].

Full substitution of the hydroxyl protons of calix[4]resorcinareneoctol (**2**) by sulfonyl groups (compound **10**) transforms the initial crown conformation into a boat conformation in which two opposite benzene rings are coplanar with the main macrocyclic plane formed by carbon atoms of the methine bridges and two others are perpendicular to the plane (C_{2v} symmetry). The boat

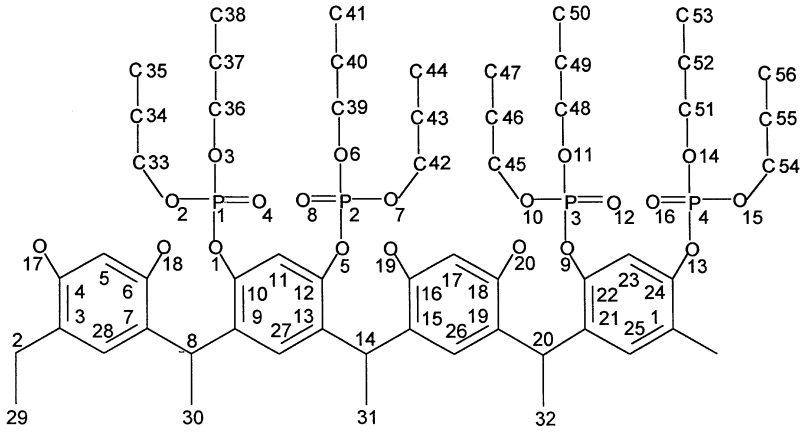


Figure 2. Numbering scheme for compound **12**

conformation is stereochemically flexible. A rapid pseudo-rotation process (Figure 1) consisting in site exchange of benzene rings with the coplanar and perpendicular orientation is observed in solutions at room temperature. The crown conformation is intermediate in this transition.[10]

In accordance with NMR data,[18,20] the boat conformation is possible also for the *O,O,O,O*-tetrasulfonylated and tetraphosphorylated derivatives **6–9, 11** and **12**. In this work an x-ray investigation of **12** was performed to obtain a more detailed representation of the spatial structure of the tetrasubstituted calixarenes.

Crystal structure of *12*

X-ray diffraction studies showed that **12** (Figure 2) in the crystal exists in a boat conformation, having one of the propyl chains self-included in the cavity (Figure 3). In this conformation, two opposite non-substituted aryl rings are almost coplanar having a dihedral angle of 15.7(1)°, while the phosphorylated rings are almost cofacial [dihedral angle = 3.2(2)°]. The first two resorcinol rings are twisted with respect to the reference plane (defined by the C atoms in the methylene bridges) by angles of 1.77(6)° and 14.79(8)°. Those 'perpendicular' to the plane intersect it at angles of 88.1(1)° and 88.9(1)°. The closest distance between them is 5.043(5) Å. For neighboring aryl rings the dihedral angles are close to a right-angle, having values of 89.7(1)°, 88.3(1)°, 88.5(1)° and 87.4(1)°. Methyl substituents at the methylene bridges all adopt sterically less strained axial positions, thus leading to an all-*cis* configuration. All this defines the symmetry of the macrocycle backbone as close to C_{2v}; however, the molecule as a whole is asymmetric owing to the asymmetric orientation of the phosphory-

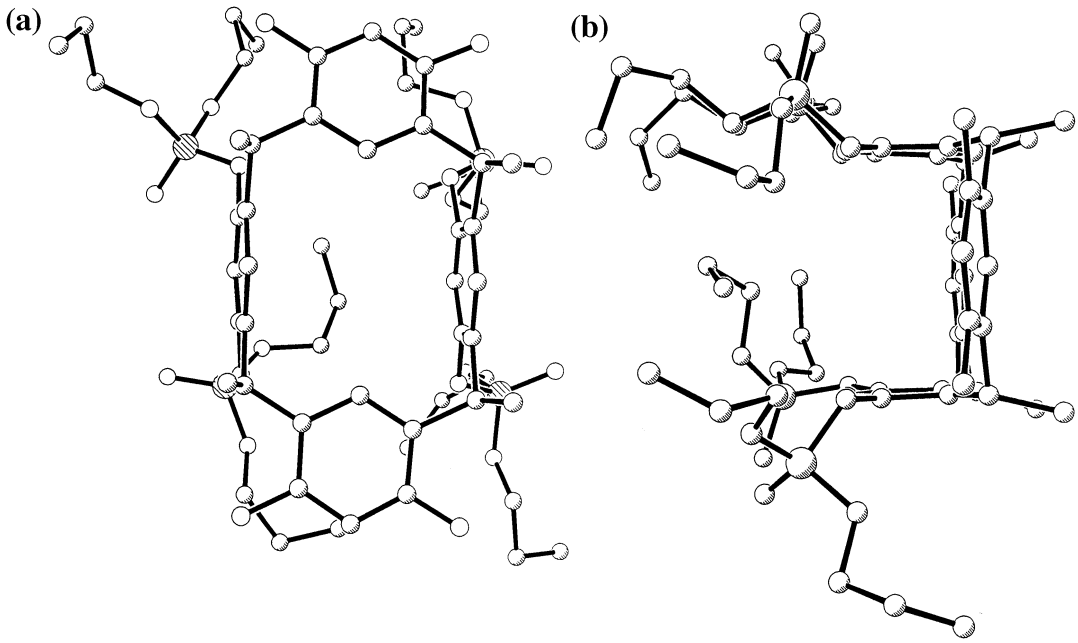


Figure 3. Molecular structure of tetrakis(dipropoxyphosphoryloxy)calix[4]resorcinarene (**12**): (a) bottom view; (b) side view

Table 2. Selected bond lengths (Å) and angles (°) for compound **12**

P(1)—O(1)	1.554(3)	O(3)—P(1)—O(1)	102.2(2)
P(1)—O(2)	1.569(4)	O(3)—P(1)—O(2)	104.8(2)
P(1)—O(3)	1.535(3)	O(1)—P(1)—O(2)	108.5(2)
P(1)—O(4)	1.438(3)	O(4)—P(1)—O(2)	108.9(2)
P(2)—O(5)	1.569(3)	O(4)—P(1)—O(3)	117.1(2)
P(2)—O(6)	1.529(3)	O(4)—P(1)—O(1)	114.6(2)
P(2)—O(7)	1.570(4)	O(5)—P(2)—O(7)	100.3(2)
P(2)—O(8)	1.460(3)	O(6)—P(2)—O(5)	103.9(2)
P(3)—O(9)	1.562(3)	O(6)—P(2)—O(7)	109.0(2)
P(3)—O(10)	1.558(4)	O(8)—P(2)—O(6)	111.9(2)
P(3)—O(11)	1.554(3)	O(8)—P(2)—O(5)	115.1(2)
P(3)—O(12)	1.441(4)	O(8)—P(2)—O(7)	115.5(2)
P(4)—O(13)	1.572(3)	O(11)—P(3)—O(9)	102.3(2)
P(4)—O(14)	1.545(4)	O(10)—P(3)—O(9)	103.4(2)
P(4)—O(15)	1.544(4)	O(11)—P(3)—O(10)	107.6(2)
P(4)—O(16)	1.420(4)	O(12)—P(3)—O(11)	112.1(2)
		O(12)—P(3)—O(10)	114.6(3)
		O(12)—P(3)—O(9)	115.8(2)
		O(15)—P(4)—O(14)	100.5(3)
		O(15)—P(4)—O(13)	102.2(2)
		O(14)—P(4)—O(13)	102.0(2)
		O(16)—P(4)—O(13)	114.7(2)
		O(16)—P(4)—O(14)	116.5(3)
		O(16)—P(4)—O(15)	118.3(3)

loxy substituents. The phosphorus atoms have a distorted tetrahedral geometry. As reported by Loeber *et al.*,[31] all the P=O double bonds are oriented outwards the cavity and, as expected, are shorter than the esterified P—O bonds, being in the range 1.420(4)–1.460(3) Å (Table 2). (Full experimental details, atomic coordinates, all bond lengths and angles, and thermal parameters are deposited as supplementary material.) The P—O single bonds range from 1.529(3) to 1.572(3) Å, which can be considered as within the usual ranges for phosphorus to oxygen bond lengths in a PO_4 fragment. All O=P—O angles are tetrahedral or larger [108.9(2)-118.3(3)°], while all O—P—O angles are tetrahedral or smaller [100.3(2)–109.0(2)°]. This is in agreement with literature data for similar phosphorylated calixarenes.[32,33]

Owing to the *exo* P=O bond orientation, no intramolecular hydrogen bonds are present in the structure. This special orientation, however, promotes the formation of intermolecular hydrogen bonds leading to an interesting layered arrangement, in which each molecule is involved in eight O—H…O H-bondings (two with each of its four neighbors) (Figure 4). All of them are of P=O…H—O

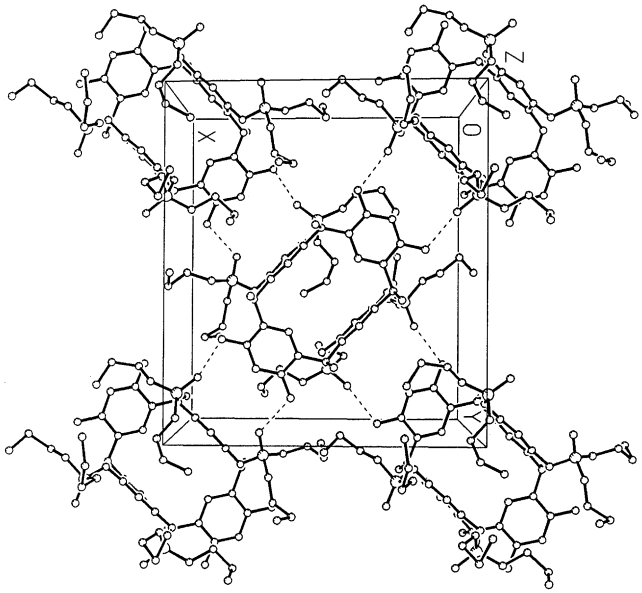


Figure 4. Packing arrangement of molecules within the unit cell and intermolecular hydrogen bonding pattern for compound **12**

TABLE 3. Geometry of the O—H…O=P hydrogen bonds for compound **12** (bond lengths in Å, angles in degrees)

	O…O	O—H	H…O	O—H…O
O(17)—H(17O)…O12[a]	2.609(4)	0.97(6)	1.65(6)	169(5)
O(18)—H(18O)…O16[a]	2.735(5)	0.78(6)	1.98(6)	163(6)
O(19)—H(19O)…O4[b]	2.719(4)	0.70(6)	2.03(6)	172(6)
O(20)—H(20O)…O8[b]	2.664(4)	0.75(6)	1.93(6)	167(7)

Symmetry transformations used to generate equivalent atoms:
[a] $-0.5-X, 0.5+Y, 0.5-Z$;
[b] $0.5-X, -0.5+Y, 0.5-Z$.

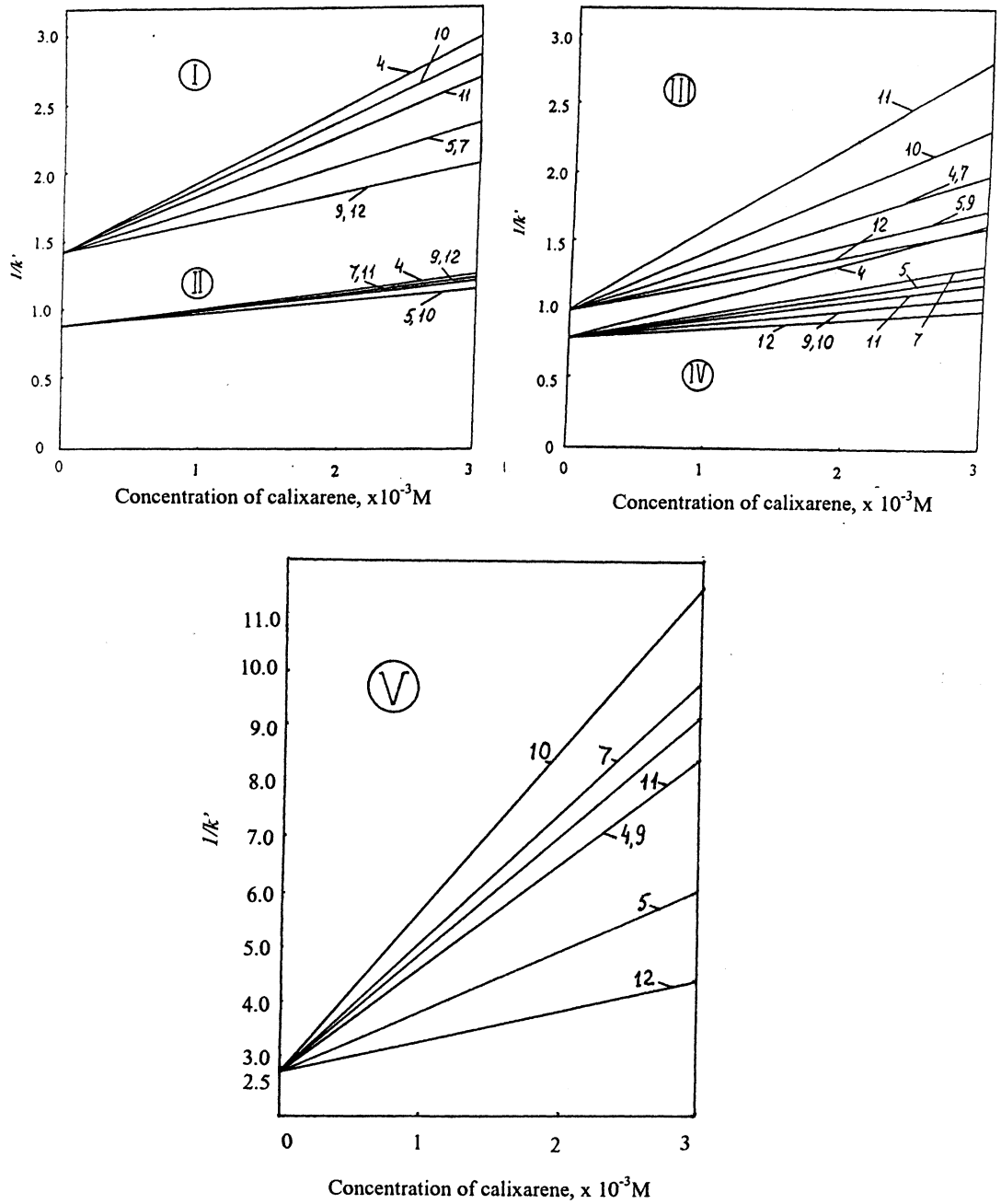


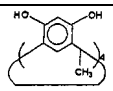
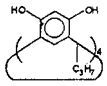
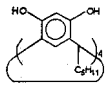
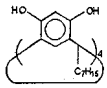
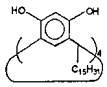
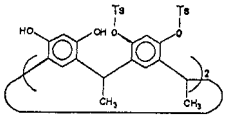
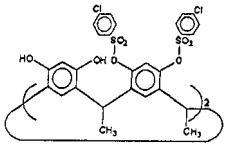
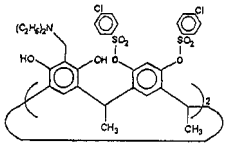
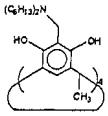
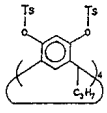
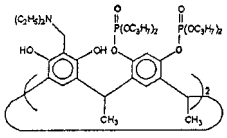
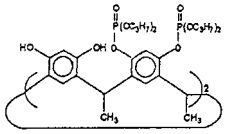
Figure 5. Plots of $1/k'$ for benzene (I), *p*-xylene (II), ethylbenzene (III), toluene (IV) and *p*-cresol (V) against concentration of calix[4]resorcinarenes **4, 5, 7,** and **9–12.** Mobile phase: solution of calix[4]resorcinarene (1×10^{-3}–3×10^{-3} M) in acetonitrile–water mixture

type, and in the molecule four are accepted by oxygens of P=O moieties and the other four are donated by the OH groups of the aryl rings. The O…O distances range from 2.609(4) to 2.735(5) Å, while the angles are 163(6)–172(6)° (Table 3).

It may be assumed that the highly asymmetric conformation of the phosphoryloxy substituents at the calixarene ring is due to the non-bonded intermolecular interactions in the solid state. From the point of view of a single molecule, one may speculate that the different conformations of all four phosphoryloxy groups indicate very significant conformational flexibility of these parts of the molecule. Such a conclusion comes also from the observed orientational disordering of two of the four lateral groups. In the absence of any guest species, the internal cavity in the calixarene molecule is filled, at least, partially by one of the lateral groups of the macrocycle.

In view of the above considerations, it might be suggested that complexation of guest species by phosphorylated calixarene host molecules is associated with conformational flexibility of the lateral substituents. This,

TABLE 4. Retention times t_R, capacity factors k' and width/height (S/h) ratios of calix[4]resorcinolarenes

Compound number	Formula	t_R	k'	S/h
1		3.67	0.84	0.04
2		6.17	2.09	0.05
3		8.33	3.17	0.02
4		11.17	4.59	0.03
5		> 80.0		
6		72.33	35.17	12.50
7		47.67	22.84	6.67
8		> 80.0		
9		72.33	35.17	12.50
10		47.67	22.84	6.67
11		> 80.0		
12		> 80.0		

in turn, may adapt the molecular structure of the host to a given guest. In such a way, complexation is selective rather than specific and may be used as a suitable basis for chromatographic separations of a range of different guest species.

HPLC investigation

Determination of retention times and capacity factors. The retention times, t_R, and capacity factors, k', for the unsubstituted tetraalkylcalix[4]resorcinareneoctols **1–5** were determined and are presented in Table 4. Dependences of the retention times and capacity factors on the number of carbon atoms in the alkyl chains are observed for all of these compounds. As shown in Table 4, the first member of the series, tetramethylcalix-[4]resorcinareneoctol (**1**), is weakly retained at the sorbent surface (t_R = 3.67 min and k' = 0.84). However, an increase in the alkyl chain length at the lower rim considerably increases the adsorption and **5,** possessing pentadecyl groups, is very strongly bonded by the sorbent surface. Replacement of the hydrogen atoms of hydroxyl groups by tosyl and *p*-chlorobenzosulfonyl substituents results in 2–7-fold increases in t_R and k'. These parameters depend on the nature and number of the substituents (Table 4). However, the most remarkable changes are observed after functionalization of the the upper rim by dipropoxyphosphoryl and *N*,*N*-dialkylaminomethyl substituents. For example, the introduction of four *N*,*N*-dihexylaminomethyl groups in **1** causes an 18-fold increase in the retention time (t_R = 72 min in **9**). Tetrakis(dipropoxyphosphoryloxy)calix[4]resorcinarene (**12**) and the diethylaminomethyl derivative **8** are also strongly bound by the sorbent surface.

The t_R and k' values obtained suggest that tetraalkylcalix[4]resorcinareneoctols **1–5** are bonded with the sorbent surface with the help of lipophilic alkyl groups on the lower rim of the macrocycle. The upper hydrophilic rim, having eight hydroxyl groups, does not participate in adsorption. Therefore, the macrocyclic cavity is opened for inclusion of a guest molecule from the mobile phase. Modification of the upper rim of the macrocycle of the weakly adsorbing tetramethyl- and tetrapropylcalix[4]resorcinarenes **1** and **2** by lipophilic arylsulfonyl, *N*,*N*-dialkylaminomethyl and dipropoxyphosphoryl substituents changes the nature of the adsorption and **6–12** are probably bonded with the sorbent surface by the upper rim of the macrocycle, which becomes more lipophilic than the lower rim.

Determination of stability constants. Detailed investigations have shown that the addition of cyclodextrins[24,25] and phenol-derived calixarenes[34] to the mobile phase leads to changes in the chromatographic characteristics of solute molecules owing to the formation of host–guest complexes. Host–guest complexation has been used in chromatographic analysis for the separation of *o*-, m- and *p*-isomers of substituted benzenes[34] and also for the separation of optical antipodes of some chiral molecules.[24,25] As with cyclodextrins[24,25] and calixarenes,[34] the introduction of calixresorcinarenes in the mobile phase decreases the retention and capacity factors of solutes (benzene, toluene, ethylbenzene, *p*-xylene and *p*-cresol) (Table 5), indicating host–guest inclusion complex formation in the acetonitrile–water solution.

To determine the composition of the complexes formed, the dependences of the $1/k'$ values of the solutes on the calixresorcinarene concentration in the mobile phase were studied. As shown in Figure 5, for all compounds investigated a linear dependence of $1/k'$ on calixarene concentration in the range 1×10^{-3}–3×10^{-3} M is observed, indicating the formation of the host–guest complexes with a 1:1 composition.[35]

From the dependences obtained of $1/k'$ on concentration of the calixarene additives, the stability constants of these complexes were calculated. The equation

$$1/k' = 1/k'_0 + [\text{host}]/K_D \times k'_0 \qquad (1)$$

where k'_0 is the capacity factor in the absence of a host, [host] is the concentration of calixarene in mobile phase and, K_D is the dissociation constant of the complex, proposed by Fujimura *et al.*[36] for the determination of the stability constants of β cyclodextrin complexes with benzene and naphthalene derivatives in methanol–water solutions, was used for the calculation.

TABLE 5. Capacity factors k' measured for aromatic solutes with using calix[4]resorcinarenes additives in eluent (MeCN-H_2O, 86:14; additives concentration 1×10^{-3} M)

	Calix[4]resorcinarenes additive number							
Solute	Control (no additive)	**4**	**5**	**7**	**9**	**10**	**11**	**12**
p-Cresol	0.39	0.22	0.28	0.20	0.22	0.17	0.21	0.30
Ethylbenzene	0.96	0.74	0.78	0.74	0.78	0.68	0.61	0.82
Benzene	0.70	0.52	0.57	0.57	0.61	0.55	0.54	0.61
Toluene	1.22	0.91	1.02	1.00	1.09	1.09	1.08	1.12
p-Xylene	1.13	1.00	1.07	1.02	1.09	1.07	1.04	1.08

In the chromatographic column containing a solute (guest) and calixarene (host), equilibria may be established between the mobile phase (m) and the stationary phase (s):

$$(\text{guest})_s \overset{K_S}{\rightleftharpoons} (\text{guest})_m + (\text{host})_m \overset{K_D}{\rightleftharpoons}$$

$$(\text{host-guest})_m \overset{K_C}{\rightleftharpoons} (\text{host-guest})_s$$

The equilibrium constants K_s, K_D and K_C are given as follows: distribution constant of guest:

$$K_S = \frac{[(\text{guest})_s]}{[(\text{guest})_m]} \tag{2}$$

dissociation constant of the host–guest complex

$$K_D = \frac{[(\text{host})_m][\text{guest})_m]}{[(\text{host-guest})_m]} \tag{3}$$

and distribution constant of host–guest complex:

$$K_C = \frac{[(\text{host-guest})_s]}{[(\text{host-guest})_m]} \tag{4}$$

In the scheme presented, the distribution of calixarene between the phases may be negligible. As shown by Table 4, calixarenes are strongly bound by the sorbent surface. Therefore, the retention of the solutes was determined after 7 h of elution of the calixarene solutions through the column. Under these conditions the column was saturated with calixarene and the host concentration in the mobile phase during the experiments was 1×10^{-3}, 2×10^{-3} and 3×10^{-3} M, as indicated in the Experimental section.

Owing to of saturation of the column with calixarene, the distribution equilibrium of the host–guest complex on to the stationary phase [equation (4)] may also be neglected (the sorption of this complex must be similar to the sorption of calixarene itself). The solute capacity factor, k', can therefore be written as

$$K' = \phi \frac{[(\text{guest})_s]}{[(\text{guest})_m] + [(\text{host-guest})]_m} \tag{5}$$

where ϕ denotes the phase ratio of the column. Considering that the total concentration of calixarene, $[\text{host}]_T$, in the mobile phase is $[\text{host}]_T = [(\text{host})_m] + [(\text{host-guest})_m]$, equation (5) may be expressed as

$$K' = \phi \frac{K_S K_D}{K_D + ([\text{host}]_T - [(\text{host-guest})_m])} \tag{6}$$

Under the condition when the solute's concentration is very low compared with the calixarene's concentration, $[\text{host}]_T - [(\text{host–guest})_m] = [\text{host}]$. Furthermore, $K_s\phi$ is equal to the capacity factor, k'_0, determined in the absence of calixarene, and therefore equation (6) may be reduced to equation (1)

As shown in Table 6, the calculated stability constants of the complexes vary in the range 30–863 M^{-1} depending on nature of the substituents on the benzene rings of the macrocyclic skeleton, the lengths of the alkyl chains on its lower rim and the nature of the aromatic guest molecules The highest stability constants were obtained for *p*-cresol (200–863 M^{-1}) and the lowest for *p*-xylene (30–91 M^{-1}).

These values may be compared with some literature data. Stability constants of 132–1148 M^{-1}, close to those in Table 6, were determined by Fujimura *et al.*[36] for complexes of several substituted phenols with β-cyclodextrin in aqueous solution. Shinkai[37] investigated complexation of a phenol-made calix[4]arene bearing sulfuric acid fragments on the upper rim with trimethylanilinium chloride in D_2O solution by an NMR titration method. Calculated from the plot of δ_{obsd} *vs* [host]/[guest], the association constant of the complex was found to be 5600 M^{-1}.

p-Sulfonatocalix[4]arene, possessing a stereochemically rigid cone-shaped molecular cavity, seems to be a more effective complexant for aromatic guests than calix[4]resocinarene derivatives existing in the boat conformation (see Figure 3).

As shown in Table 7 calix[4]resorcinarenes **4,5,7** and **9–12** as additives to the mobile phase improve the separation factors R_s and resolution α of aromatic compounds with similar properties. The best values of R_s (exceeding 1.85-fold the values obtained in blank experiments) were observed for the separation of toluene and ethylbenzene in the presence of tetraheptylcalix[4]-resorcinoreneoctol **(4).** The results obtained indicate that calix[4]resorcinarenes, similarly to cyclodextrins, could

TABLE 6. Calculated stability constants K_A for complexes of aromatic solutes with calix[4]resorcinolarenes and their derivatives (s.d. = standard deviation)

	Calix[4]resorcinolarene additive number						
Solute	**4** (s.d.)	**5** (s.d.)	**7** (s.d.)	**9** (s.d.)	**10** (s.d.)	**11** (s.d.)	**12** (s.d.)
p-Cresol	517 (3.6)	263 (0.9)	635 (1.4)	517 (4.5)	863 (1.8)	572 (4.0)	200 (3.6)
Benzene	229 (2.7)	149 (6.0)	149 (6.1)	98 (4.8)	182 (4.1)	196 (12)	98 (7.8)
Ethylbenzene	199 (3.0)	153 (4.4)	199 (3.0)	153 (5.5)	275 (3.3)	49 (4.7)	32 (6.6)
Toluene	228 (3.4)	130 (4.0)	179 (5.6)	81 (5.2)	81 (5.8)	89 (4.5)	57 (12.8)
p-Xylene	91 (5.3)	38 (12.6)	75 (19.4)	30 (7.2)	38 (6.2)	60 (4.2)	38 (8.7)

TABLE 7. Separation factors R_s and resolution α for aromatic solutes

Solute		Calix[4]resorcinolareneoctol additive number							
		Control (no additive)	**4**	**5**	**7**	**9**	**10**	**11**	**12**
Benzene/methylbenzene	R_s	1.14	1.53	1.43	1.67	1.50	1.80	1.38	1.69
	α	1.33	1.35	1.37	1.41	1.44	1.36	1.33	1.39
Ethylbenzene/toluene	R_s	1.00	1.85	1.25	1.71	1.29	1.69	1.36	1.60
	α	1.28	1.45	1.29	1.32	1.31	1.29	1.30	1.33
p-Cresol/*p*-Xylene	R_s	4.24	5.54	5.64	5.25	5.33	5.29	5.41	5.50
	α	3.69	4.27	4.33	4.45	5.00	4.60	4.53	4.38

be utilized in the liquid chromatographic analysis of aromatic compounds.

CONCLUSION

Tetraalkylcalix[4]resorcinareneoctols and their derivatives functionalized at the upper rim of the macrocycle by *N*,*N*-dialkylaminomethyl, arylsufonyl and dipropoxyphosphoryl groups form in acetonitrile–water solutions host–guest inclusion complexes with benzene derivatives. The stability constants of these complexes are 30–863 M^{-1}. As with cyclodextrins, the formation of the complexes with calix[4]resorcinarenes changes the retention of the investigated aromatic molecules on the sorbent LiChrosorb RP-18 surface and leads to an improvement of the separation of compounds possessing similar properties. This phenomenon could be used in applied chromatography.

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