Effect of Octakis(diethoxyphosphoryloxy)-*tert*-butyl-calix[8]arene in Mobile Phase on the Reversed-Phase Retention Behavior of Aromatic Compounds: Host–Guest Complex Formation and Stability Constants Determination

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Abstract

Under reversed-phase high-performance liquid chromatographic conditions (Separon SGX C_{18} stationary phase, ultraviolet detection at 254 nm, and acetonitrile–water [86:14, v/v] as the mobile phase), addition of octakis(diethoxyphosphoryloxy)-*tert*-butyl-calix[8]arene in concentrations of 1×10^{-4} to 4×10^{4}M to the mobile phase leads to decreasing sorption of aromatic solutes on the sorbent's surface due to the formation of host–guest inclusion complexes between the calixarenes and the aromatic molecules. Stability constants of the complexes (608–2795M^{-1}) are determined from the relationship between the solute capacity factors and the calixarene concentration in the mobile phase.

Introduction

Macrocyclic compounds (cyclodextrins [1], calixarenes, and calixresorcinarenes [2–4]) possessing molecular cavities are well-known host molecules capable of forming host–guest complexes with a variety of organic (5,6) and inorganic (4) guests in solution (7), solid state (8), and thin films (9). Consequently, macrocyclic compounds have been extensively employed as additives in the mobile phase or as a stationary phase in both gas and liquid chromatography (LC) (10,11). For example, cyclodextrins have been used for the separation of *o*-, *m*-, and *p*- isomers of benzene derivatives, resolution of chiral compounds into enantiomers (10), and drug analysis (12). Crown ethers have been successfully used for the separation of saccharides in pulp effluents (13).

The utility of water-soluble calix[6]arene–*p*-sulfonate as a mobile phase additive has been investigated for the reversed-phase LC separation of some monosubstituted phenol isomers (14). Recently (15), chromatographic behavior of some calix[4]-, calix[6]-, and calix[8]arenes functionalized at the lower rim of the macrocyclic skeleton by phosphoryl groups under reversed-phase high-performance liquid chromatographic (HPLC) conditions has been examined in detail.

In this work, we reported the effect of adding octakis(diethoxyphosphoryloxy)-*tert*-butyl-calix[8]arene (CA) (see Figure 1), which has a molecular cavity diameter (1.17 nm) similar to that

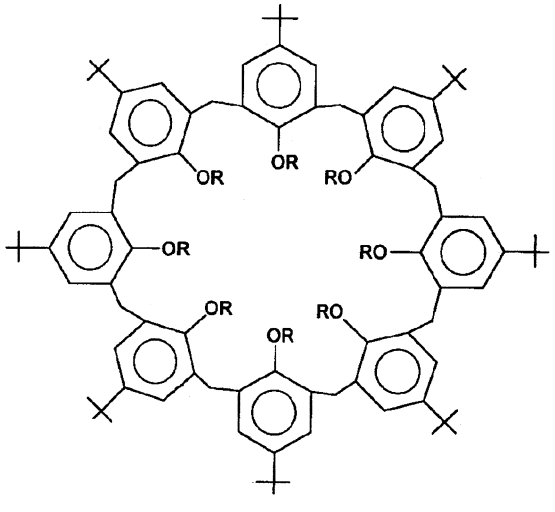

Figure 1. Structural formula of the title calixarene.

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of β-cyclodextrin (0.78 nm), to an acetonitrile–water mobile phase on the reversed-phase retention behavior of aromatic molecules. The stability constants of host–guest inclusion complexes of CA with aromatic molecules were determined from the relationship of the guest retention value and concentration of CA in the mobile phase.

Experimental

Apparatus

The LC system consisted of a high-pressure pump HPP 4001 (Laboratorni Pristroje, Praha, Czechoslovakia) connected to a Rheodyne sample 7120 injector with a 20-µL loop (Rheodyne, Berkeley, CA) and an ultraviolet–visible (UV–vis) detector LCD 2563 (Laboratorni Pristroje, Praha, Czechoslovakia). The column (150 × 3.3-mm i.d.) was packed with Separon SGX C_{18} (5 µm) (Lachema, Czechoslovakia).

Reagents

All sample compounds were of the best quality available and were purchased from various suppliers. The 49,50,51,52,53,54, 55,56-octakis-(diethoxyphosphoryloxy)-*tert*-butyl-calix[8]arene was prepared by reaction of the initial *tert*-butyl-calix[8]areneoctol with diethylchlorophosphate in a method similar to that described in the literature (16), but sodium hydride was used as the base. A suspension of 2.53 g (1.95mM) *tert*-butyl-calix[4]areneoctol and 0.75 g (31mM) sodium hydride in 100 mL dry tetrahydrofuran (THF) was refluxed for 0.5 h, and 6.7 g (39mM) diethylchlorophosphate was added. The reaction mixture was refluxed for 4 h and evaporated at a reduced pressure. The residue (viscous oil) was recrystallyzed from *n*-heptane and purified by column chromatography (silicagel; eluent mixture, benzene–acetone [1:1]). A 2.1-g amount (yield, 48%) of phosphorylated calix[8]arene was obtained (melting point, 202–205°C) (recrystallized from heptane).

The nuclear magnetic resonance spectra were as follows: ^1H NMR (300 MHz, $CDCl_3$) δ 6.95 (*s*, 16H, Harom), 4.20 (*s*, 16H, Ar_2CH_2), 3.96 (*m*, 32H, OCH_2CH_3), 1.06 (*t*, 48H, J = 7 Hz, OCH_2CH_3), 1.01 (*s*, 72H, *t*-C_4H_9); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$) δ 147.19 (*d*, J_{CP} = 2 Hz), 145.21 (*d*, J_{CP} = 8 Hz), 131.61 (*d*, J_{CP} = 3.2 Hz), 126.54, 64.34 (two *d*, J_{CP} = 6 Hz), 34.12, 31.42, 31.24, 15.89 (four *d*, J_{CP} = 6.8 Hz); ^{31}P NMR (120 MHz, $CDCl_3$) δ 4.0. Elemental analysis provided the following results: C, 60.17; H, 7.66 (calculated for $C_{120}H_{194}O_{32}P_8$; C, 60.39; H, 7.77).

HPLC analysis

The mobile phase (an acetonitrile–water eluent containing calix[8]arene at concentrations of 1×10^{-4}, 2×10^{-4}, 3×10^{-4}, and 4×10^{-4}M) was prepared by dissolving the calixarene in an acetonitrile–water (86:14, v/v) solution at ambient temperature. Each of the four concentrations was analyzed five times. Sample solutions for injections were prepared so as to give a concentration of 0.5×10^{-4}M for each solute (S) using a solvent identical to the mobile phase. The amount of the sample injected was 20 µL. Each of the samples was analyzed five times. All chromatograms were obtained at 24°C. The flow rate was 0.5 mL/min, and the UV detector was operated at 254 nm. The dead time (t_0) was measured with $NaNO_2$.

Results and Discussion

Effect of the CA mobile phase additive on retention of the aromatic solutes

Numerous studies of cyclodextrins as mobile phase additives in LC have demonstrated that the retention time of a solute usually becomes shorter in the presence of the macrocycles. This has been explained by the host–guest inclusion complex formation, which weakens the interaction of the solute with the stationary phase. Increased selectivity due to the formation of the complexes has also been observed in many cases, but not necessarily in all. A similar reduction of the retention time as well as increased selectivity were demonstrated in the work (14) in which water-soluble *p*-sulfonatocalix[6]arene host molecules were used for the reversed-phase LC separation of some monosubstituted phenol isomers.

The influence of the CA additive to the mobile phase on retention time (t_R) and capacity factor (k') of 20 different benzene

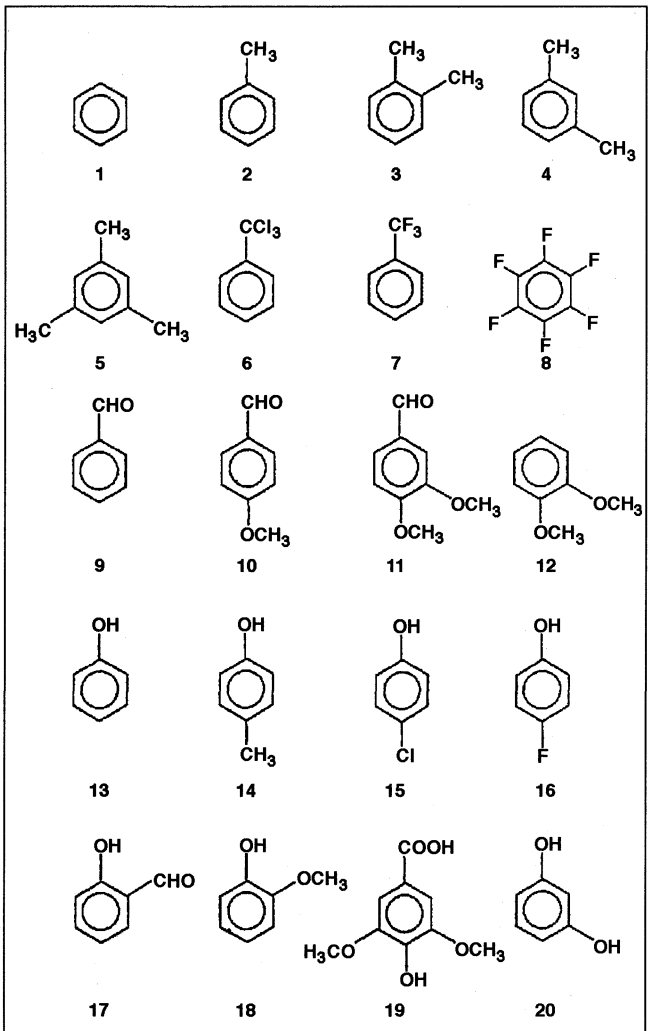

Figure 2. Structural formulas of guests 1–20.

derivatives (Figure 2) was studied. Investigated compounds contain substituents at the benzene ring that differ in size and nature: fluorine and chlorine atoms, methyl, trifluoro-(trichloro)methyl, methoxy, hydroxy, formyl, and carboxyl groups. These substituents determine capacity factors of 20 compounds that lie in a wide region from 0.17 for syringic acid 19 to 2.90 for 1,3,5-trimethylbenzene 5 (Table I).

As shown in Table I, the capacity factor of solutes 1-20 was decreased by the CA additive. These results clearly confirm formation of CA–S host–guest complexes in the mobile phase. Like cyclodextrins, the CA complexation weakened the interaction between the aromatic solute and the Separon SGX C_{18} stationary phase, decreasing the capacity factor. Because the change of values caused by the host–guest complexation was dependent on CA concentration, it was possible to determine the stability constants of the complexes formed.

Stability constant determination

The stability constants of the calixarene complexes with neutral organic molecules in solutions are usually determined by NMR spectrometry (2–7). For similar cyclodextrin complexes, however, a number of convenient chromatographic procedures for stability constant determination were developed (10–12, 17,18). These procedures are based on the relationship between solute retention parameters and cyclodextrin concentration in the mobile phase. Taking into account the change in the solute capacity factors influenced by the addition of CA in the mobile phase, this method may be used for the determination of stability constants. The variation of the capacity factors of compounds 1–20 with increasing CA concentration in the mobile phase (0, 1×10^{-4}, 2×10^{-4}, 3×10^{-4}, and 4×10^{-4}M) is presented in Table I and Figure 3. Among the chromatographic determinations of host–guest stability constants described in the literature (10–12,17,18), Fujimura's method (17) was chosen for the investigation of CA complexes with aromatic guests.

In the chromatographic column containing S and CA, the following equilibria may be established between the mobile phase (m) and the stationary phase (s)

$$(S)_s \overset{K_S}{\rightleftarrows} (S)_m + (CA)_m \overset{K_D}{\rightleftarrows} (CA\text{–}S)_m \overset{K_C}{\rightleftarrows} (CA\text{–}S)_s$$

The equilibrium constants K_S, K_D, and K_C are given as follows:

Distribution constant of solute S:

$$K_S = \frac{[(S)_s]}{[(S)_m]} \qquad \text{Eq 1}$$

Dissociation constant of the complex CA–S:

$$K_D = \frac{[(CA)_m][(S)_m]}{[(CA\text{–}S)_m]} \qquad \text{Eq 2}$$

Distribution constant of CA–S:

$$K_C = \frac{[(CA\text{–}S)_s]}{[(CA\text{–}S)_m]} \qquad \text{Eq 3}$$

In the proposed scheme, the distribution of CA between the phases may be negligible. Under the analysis conditions, the

Table I. Effect of Mobile Phase Calix[8]arene Concentration on Capacity Factors*

	k' ($\Delta k'$)†				
Substance	No additive (reproducibility [k', %])	1×10^{-4}M	2×10^{-4}M	3×10^{-4}M	4×10^{-4}M
1. Benzene	1.08 (0.93)	0.92 (–0.15)	0.81 (–0.25)	0.72 (–0.33)	0.66 (–0.38)
2. Toluene	1.12 (0.89)	1.03 (–0.08)	0.95 (–0.15)	0.91 (–0.18)	0.90 (–0.19)
3. *o*-Xylene	2.40 (0.84)	2.0 (–0.17)	1.70 (–0.29)	1.46 (–0.42)	1.33 (–0.45)
4. *m*-Xylene	2.40 (0.41)	2.10 (–0.13)	1.70 (–0.29)	1.46 (–0.42)	1.33 (–0.45)
5. 1,3,5-Trimethylbenzene	2.90 (1.03)	2.50 (–0.14)	2.04 (–0.18)	1.82 (–0.37)	1.72 (–0.41)
6. Trichloromethylbenzene	2.75 (0.73)	2.62(–0.05)	2.41 (–0.12)	2.34 (–0.15)	2.25 (–0.18)
7. Trifluoromethylbenzene	2.13 (0.95)	1.81 (–0.15)	1.58 (–0.26)	1.39 (–0.35)	1.17 (–0.45)
8. Hexafluorobenzene	1.50 (2.05)	1.28 (–0.15)	1.09 (–0.27)	1.02 (–0.32)	0.89 (–0.41)
9. Benzaldehyde	1.08 (0.93)	0.92 (–0.15)	0.81 (–0.25)	0.72 (–0.33)	0.66 (–0.38)
10. Anisaldehyde	1.12 (2.65)	1.03 (–0.08)	0.95 (–0.15)	0.91 (–0.18)	0.90 (–0.19)
11. Veratraldehyde	1.13 (2.67)	1.0 (–0.12)	0.91 (–0.19)	0.83 (–0.27)	0.71 (–0.37)
12. Veratrole	1.08 (2.82)	0.90 (–0.17)	0.81 (–0.25)	0.71 (–0.34)	0.70 (–0.35)
13. Phenol	1.08 (2.82)	0.90 (–0.17)	0.78 (–0.28)	0.68 (–0.37)	0.54 (–0.50)
14. 4-Methyl phenol	1.12 (2.93)	0.90 (–0.20)	0.75 (–0.33)	0.65 (–0.42)	0.57 (–0.49)
15. 4-Chloro phenol	1.12 (3.64)	0.90 (–0.20)	0.76 (–0.32)	0.64 (–0.43)	0.63 (–0.44)
16. 4-Fluoro phenol	1.03 (3.74)	0.90 (–0.13)	0.78 (–0.24)	0.76 (–0.26)	0.67 (–0.35)
17. Salicyl aldehyde	0.75 (2.63)	0.70 (–0.07)	0.65 (–0.13)	0.60 (–0.20)	0.53 (–0.29)
18. Guaiacol	1.05 (2.90)	0.90 (–0.14)	0.79 (–0.25)	0.70 (–0.33)	0.59 (–0.44)
19. Syringic acid	0.17 (3.07)	0.14 (–0.18)	0.13 (–0.24)	0.11 (–0.35)	0.10 (–0.35)
20. Resorcinol	1.0 (4.08)	0.78 (–0.22)	0.68 (–0.32)	0.51 (–0.49)	0.47 (–0.53)

* Column, Separon SGX C_{18} (15 cm × 3.3-mm i.d.); mobile phase, acetonitrile–water (86:14, v/v); flow rate, 0.5 mL/min.
† $\Delta k'$ indicates the variation of k' defined as $\Delta k' = (k'_{add.} - k'_{no\ add.}) / k'_{no\ add.}$

capacity factor of CA was 9.39. Therefore retention of the solutes was determined after 7 h of eluting the CA solutions through the column. Under these conditions, the column was saturated with CA, and its concentration in the mobile phase was the same as indicated in Table I.

Due to saturation of the column with CA, the distribution equilibrium of the CA–S complex onto the stationary phase (Equation 3) may also be neglected (the sorption of this complex must be similar to sorption of CA itself). The solutes capacity factor can therefore be written as

$$k' = \phi \frac{[(S)_s]}{[(S)_m] + [(CD\text{–}S)_m]} \qquad \text{Eq 4}$$

where ϕ denotes the phase ratio of the column. The total concentration of calixarene ($[CA]_T$) in the mobile phase consists of the following

$$[CA]_T = [(CA)_m] + [(CA\text{–}S)_m] \qquad \text{Eq 5}$$

Therefore, Equation 4 may be expressed as

$$k' = \phi \frac{K_S K_D}{K_D + ([CA]_T - [(CA\text{–}S)_m])} \qquad \text{Eq 6}$$

When the solute concentration is very small compared with the calixarene's concentration,

$$[CA]_T - [(CA\text{–}S)_m] = [CA] \qquad \text{Eq 7}$$

Furthermore, $K_S\phi$ is equal to the capacity factor (k'_0) determined in the absence of CA. Therefore Equation 6 may be reduced to

$$\frac{1}{k'} = \frac{1}{k'_0} + \frac{[CA]_T}{K_D k'_0} \qquad \text{Eq 8}$$

The straight linear relationship of $1/k'$ versus the calixarene concentration ($[CA]_T$) (Figure 2) confirms formation of the complexes with a 1:1 stoichiometry (18) for all the compounds investigated.

The stability constants ($K_A = 1/K_D$) of the complexes calculated from Equation 8 were within the range 608–2794M^{-1} (Table II). These constants agree with the literature data. Shinkai (3) has investigated the complexation of *p*-sulfonatocalix[8]arene (host) with trimethylanilinium chloride (guest) in a D_2O solution by NMR titration. Calculated from the plot of δ_{obsd} versus host/guest, the association constant of the complex with a 1:1 stoichiometry was 5200M^{-1}. The constants (132–1148M^{-1}), which are close to those presented in Table II, were determined by Fujimura (17) for complexes of several substituted phenols with β-cyclodextrin in a water solution.

Analysis of the stability constants (K_A) indicated that binding of the aromatic guest molecules was influenced by their size and hydrophobicity and the presence of protonodonative groups that can form hydrogen bonds with oxygen atoms of CA phosphoryl groups as well as CH-π bonds with the macrocyclic skeleton benzene rings.

The highest stability constant (2795M^{-1}) was obtained for the complex of resorcinol 20, which can form hydrogen bonds simultaneously with two phosphoryl groups of CA. The lowest constant (608M^{-1}) was observed for the trichloromethylbenzene

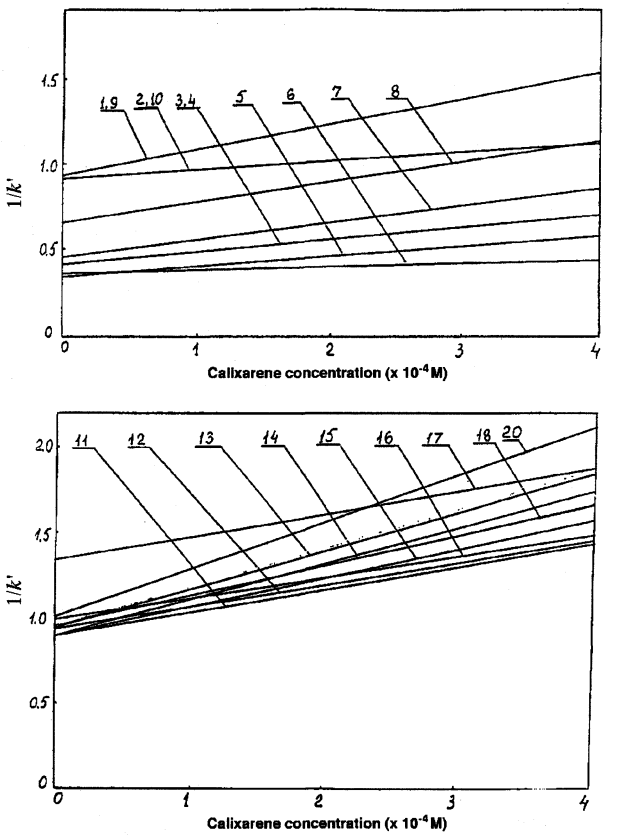

Figure 3. Plots of $1/k'$ versus calixarene concentration. Column, Separon SGX C_{18}, 15 cm x 3.3-mm i.d.; flow rate, 0.5 mL/min; detection, 254 nm; acetonitrile–water (86:14, v/v) containing 1 x 10^{-4} to 4 x 10^{-4}M calix[8]arene additive. Correlation coefficients were between 0.9962 and 0.9999.

Table II. Stability Constants of Calix[8]arene Complexes with Benzene Derivatives Measured in MeCN–H_2O (86:14, v/v) at 24°C

Compound	K_A (M^{-1}) (A ± *s*)	RSD (%)
1. Benzene	1475 ± 144.25	9.78
2. Toluene	2287 ± 186.62	8.16
3. *o*-Xylene	2073 ± 44.57	2.15
4. *m*-Xylene	1885 ± 259.75	13.78
5. 1,3,5-Trimethylbenzene	1921 ± 108.73	5.66
6. Trichloromethylbenzene	608 ± 34.47	5.67
7. Trifluoromethylbenzene	1802 ± 76.04	4.22
8. Hexafluorobenzene	1691 ± 67.98	4.02
9. Benzaldehyde	1649 ± 83.44	5.06
10. Anisaldehyde	797 ± 66.23	8.31
11. Veratraldehyde	1328 ± 64.54	4.86
12. Veratrole	1653 ± 135.71	8.21
13. Phenol	2066 ± 137.60	6.66
14. 4-Methylphenol	2441 ± 12.45	0.51
15. 4-Chlorophenol	2305 ± 233.50	10.13
16. 4-Fluorophenol	1386 ± 90.23	6.51
17. Salicyl aldehyde	858 ± 65.81	7.67
18. Guaiacol	1745 ± 66.66	3.82
19. Syringic acid	1813 ± 124.73	6.88
20. Resorcinol	2795 ± 173.85	6.22

6 complex. This host molecule cannot form any hydrogen or CH-π bonds. On the other hand, the bulky trichloromethyl group restricted inclusion of the molecule in the CA cavity. Solvatophobic forces may play the main role in the binding of trichloromethylbenzene. The nature of the observed host–guest interactions seems complicated, and additional experiments are needed for their detailed investigation.

Conclusion

Calix[8]arene has eight diethoxyphosphoryl groups at the macrocyclic rim, and like *p*-sulfonatocalix[8]arene and β-cyclodextrin, it forms stable host-guest complexes with different benzene derivatives in a water system. Reversed-phase HPLC is a useful tool for determining the stability constants of these complexes.

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