Investigation of Host–Guest Stability Constants of Calix[*n*]arenes Complexes with Aromatic Molecules by RP-HPLC Method

R. Baudry[1], O. Kalchenko[2], I. Dumazet-Bonnamour[1], F. Vocanson[1], and **R. Lamartine[1,*]**

[1]Laboratoire de Chimie Industrielle, CNRS-UMR 5078, Université Claude Bernard–Lyon1, 43, Bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France and [2]Institute of Organic Chemistry, Ukrainian Academy of Sciences, 25660 Kiev-94, Ukraine

Abstract

Under reversed-phase high-performance liquid chromatographic conditions [Spherisorb ODS 1 stationary phase, UV detection at 254 nm, and acetonitrile–dichloromethane–acetic acid–methyl-*tert*-butylether (84.6/4.5/0.9/10, v/v/v/v) as the mobile phase], adding *p-tert*-butylcalix[8] (10^{-5}–3.10^{-5})–[12]arenes (10^{-5}–4.10^{-5} mol/L) to the mobile phase leads to decreased sorption of aromatic solutes on the surface of the sorbent because of the formation of host–guest inclusion complexes between the calixarenes and the aromatic molecules. Stability constants of the complexes (781–9338M^{-1}) are determined from the relationship between the solute capacity factors and the calixarene concentration in the mobile phase.

Introduction

Calixarenes are cavity-shaped cyclic molecules made up of phenol units linked via methylene groups, which are able to complex ions (1) or neutral molecules (1–4). According to the size of the calixarene, the cavity assumes a wide variety of shapes (5). The *p-tert*-butylcalix[4]arene, which has received the most attention since the beginning of the chemistry of calixarenes, can afford four different conformations (cone, partial cone, 1-2 alternate, and 1-3 alternate). In the cone conformation, complexation of nonionic guests has been observed in the solid phase (1), solution (6), and gas phase (7). The chemical nature of the cavity and its geometry seem essential for calix[4]arene inclusion complex formation. In this work, we tried not to vary the chemical nature of the cavity, but only influence the size of *p-tert*-butylcalix[*n*]arenes. Because of the difficulties of isolating and purifyong large calixarenes (more than 8 aryl units), their host–guest complexation properties have not been extensively studied (8–10). In this paper, results of the complexation study of neutral molecules by *p-tert*-butylcalix[4–12]arenes and *p-tert*-butyldihomooxocalix[4]arene by a reversed-phase (RP) high-performance liquid chromatography (HPLC) procedure are reported.

* Author to whom correspondence should be addressed: email r.lamartine@cdlyon.univ-lyon1.fr.

Experimental

Materials

All sample compounds were of the highest quality available and were purchased from Aldrich (St. Quentin Fallavier, France) and ACROS (Noisy le Grand, France). Calixarenes were prepared by reacting *p-tert*-butylphenol using a method similar to that described in the literature (11–16). The acetonitrile, dichloromethane, acetic acid, and methyl-*tert*-butylether used in the mobile phase were HPLC solvent-grade from SDS (Peypin, France). Mixtures of solvents were filtered through a membrane filter of 0.2-µm pore size (Whatman, Maidstone, U.K.) and were degassed with a nitrogen flow.

Apparatus

The instruments consist of two Kontron (Milan, Italy) Model 322 system pumps, a Rheodyne (Cotati, CA) injector with a sample loop of 20 µl, a Kontron Model 430 UV detector coupled with a Kontron Model Data 450 PC integration pack. The sam-

Table I. Composition of Solution A

Calixarene	Acetonitrile (%, v/v)	Dichloromethane (%, v/v)	Acetic acid (%, v/v)
p-tert-Butylcalix[4–7, 9–11]arene and *p-tert*-Butyldihomooxocalix[4]arene	94	5	1
p-tert-Butylcalix[8]arene	92.66	6.36	0.98
p-tert-Butylcalix[12]arene	92.37	6.64	0.98

ples were analysed on a 250- × 4.6-mm column packed with Spherisorb ODS 1 (5 µm, Touzard and Matignon, Courtaboeuf, France). The absorbancies were measured at 281 nm [determination of calixarenes retention times (t_R)] and 254 nm (study of complexation with aromatic guests) with a 0–0.05 absorbance range. The elution was isocratic, and the temperature was ambient. The flow rate was 0.8 mL/min. The mobile phase consisted of 90% solution A (Table I)–10% methyl-*tert*-butylether. Because of the lack of solubility of *p-tert*-butylcalix[8,12]arenes, a modified mobile phase had been used. In these conditions (17), calix[4–12]arenes (even in a synthetic mixture) are able to be eluted and separated in a satisfactory manner. The t_R and capacity factors (k') of calixarenes are recorded in Table II (the solutions were prepared by dissolving calixarenes in dichloromethane at a concentration of 2.5×10^{-3}M). Whenever the mobile phase solution was changed, the column was conditioned for at least 20 min with the new solution mixture.

Range of concentration

In order to study the influence of the calixarene additive in the mobile phase on t_R, different types of aromatic derivates were used: (*a*) phenols, (*b*) polycyclic aromatic hydrocarbons (PAHs), and (*c*) thioether aromatic compounds (only with *p-tert*-butylcalix[8–12]arenes). Guest concentrations were chosen so that the response of the detector was equivalent for all solutes when used without calixarene in the mobile phase (Table III).

Table II. Chromatographic Characteristics of Calixarenes

Calix[*n*]arene	Symbol	t_R (min)	Capacity factor (k')
4	4+	4.86	0.53
5	5+	8.01	1.44
6	6+	7.94	1.4
7	7+	9.81	1.8
8	8+	10.32	1.93
9	9+	11.84	2.56
10	10+	12.31	2.68
11	11+	18.82	4.41
12	12+	23.25	5.80
Dihomooxo calix[4]arene	4(0)	5.37	0.69

Table III. Solutes and their Concentrations

N°	Guest	Concentration (M^{-1})
11	Carbetamide *N*-ethyl-2-(phenylcarbamoyloxy)propionamide	7.5×10^{-6}
12	Anthracene	7.4×10^{-6}
13	Naphthalene	1.3×10^{-4}
14	Thianaphthene	3.26×10^{-5}
15	*m*-Xylene	1.1×10^{-3}
16	*p-tert*-Butylphenol	3.6×10^{-4}
17	Pentachlorophenol	7.4×10^{-5}
18	Fluoranthene	7.3×10^{-6}
19	Dibenzothiophene	2.92×10^{-5}

Moreover, samples had to be sufficiently concentrated to be detected. Usually, dead volume of the column is determined from $NaNO_2$ retention, but the nonaqueous mobile phase system does not permit solubilization of this salt. Therefore, samples of solutes were prepared with a mobile phase customized with an excess of dichloromethane. The t_R of dichloromethane has been considered as the dead volume of the column. Each of the samples was analyzed three times.

The *p-tert*-butylcalix[4–12]arenes and *p-tert*-butyldihomooxocalix[4]arene were used as host molecules. They were introduced in the mobile phase at the following concentrations: 9.10^{-6}, 2.10^{-5}, 3.10^{-5}, and 4.10^{-5} mol/L. Only three concentrations were used with *p-tert*-butylcalix[8,9]arenes.

Results and Discussion

Effect of the calixarene mobile phase addition on the retention of the aromatic solutes

Numerous studies of cyclodextrins (18–21) as mobile phase additives in liquid chromatography (LC) have demonstrated that the t_R of a solute usually becomes shorter in the presence of the macrocycle additive. This has been explained by the host–guest inclusion complex formation, which weakens the interaction of the solute with the stationary phase. The formation of the complex can also increase the selectivity. Such behaviors have already been observed in cases using calixarene (22–26) or resorcinarene (27). Typically, adding calix[6]arene-*p*-sulfonate to both acetonitrile–water and methanol–water caused a reduction in the retention of phenol (26) isomers and generally increased the

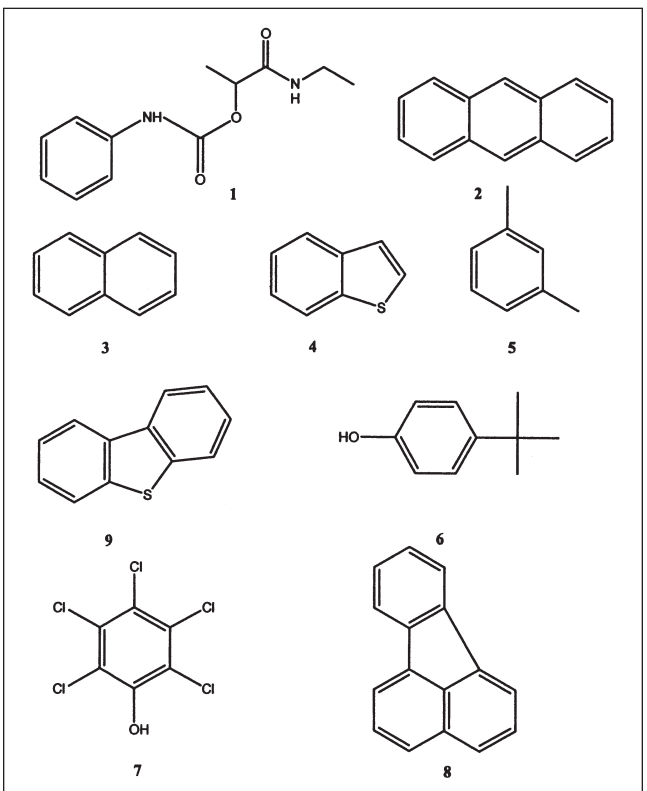

Figure 1. The structural formulas of solutes.

separation between them.

The influence of the calixarene additive to the mobile phase on the t_R and capacity factor of nine various aromatic molecules (Figure 1) was studied. Investigated compounds differed from the number of aromatic rings and from the substituants intra- or extraring.

As shown in Tables IV and V, the capacity factor of solutes was decreased by the calixarene additive. These results clearly

Table IV. Effect of Mobile Phase *p-tert*-Butylcalix[*n*]arene Concentration on Capacity Factors (k')*

		k'				
Substance	**Calixarene**	**No additive**	**9.10^{-6}M**	**2.10^{-5}M**	**3.10^{-5}M**	**4.10^{-5}M**
Anthracene	10+	0.241	0.242	0.24	0.236	0.235
	12+	0.238	0.234	0.23	0.227	0.225
Naphthalene	9+	0.139		0.134	0.135	0.132
	12+	0.138	0.134	0.129	0.127	0.125
Thianaphthene	11+	0.116	0.114	0.112	.011	0.108
m-Xylene	8+	0.144	0.133	0.134	0.128	
	10+	0.148	0.149	0.142	0.139	0.14
	11+	0.148	0.143	0.144	0.136	0.136
	12+	0.146	0.142	0.137	0.132	0.132
p-tert-Butylphenol	8+	0.086	0.075	0.071	0.066	
Pentachlorophenol	8+	0.284	0.247	0.236	0.227	
	9+	0.307		0.287	0.284	0.284
	11+	0.307	0.29	0.286	0.276	0.274
Fluoranthene	9+	0.299		0.289	0.283	0.286
	11+	0.299	0.29	0.284	0.278	0.278
Dibenzothiophene	12+	0.228	0.222	0.227	0.217	0.214

* Correlation coefficient greater than 9 for $1/k'$ versus calixarene concentration.

Table V. Effect of Mobile Phase *p-tert*-Butylcalix[*n*]arene Concentration on Capacity Factors (k')*

		k'				
Substance	**Calixarene**	**No additive**	**9.10^{-6}M**	**2.10^{-5}M**	**3.10^{-5}M**	**4.10^{-5}M**
Carbetamide	8+	0.205	0.194	0.189	0.192	
	12+	0.205	0.191	0.199	0.186	0.184
Anthracene	8+	0.24	0.206	0.208	0.203	
	11+	0.241	0.244	0.242	0.233	0.234
Naphthalene	8+	0.138	0.122	0.123	0.12	
	11+	0.139	0.143	0.139	0.132	0.131
Thianaphthene	8+	0.116	0.107	0.105	0.105	
	12+	0.12	0.11	0.111	0.108	0.106
m-Xylene	9+	0.148		0.145	0.142	0.144
p-tert-Butylphenol	10+	0.083	0.086	0.08	0.075	0.078
	11+	0.083	0.081	0.083	0.076	0.075
	12+	0.085	0.078	0.072	0.072	0.072
Pentachlorophenol	10+	0.307	0.284	0.28	0.276	0.294
	12+	0.286	0.269	0.258	0.26	0.261
Fluoranthene	8+	0.263	0.253	0.233	0.251	
	10+	0.299	0.289	0.285	0.285	0.3
Debenzothiophene	8+	0.225	0.208	0.196	0.202	
	11+	0.229	0.234	0.228	0.223	0.219

* Correlation coefficient greater than 0.8 (< 0.9) for $1/k'$ versus calixarene concentration.

confirm the formation of calixarene–solute host–guest complexes in the mobile phase. Like cyclodextrins, the calixarene complexation weakened the interaction between the aromatic solute and the stationary phase, which decreased the capacity factor. Because the change of values caused by the host–guest complexation was dependent on calixarene concentration, it was possible to determine the stability constants of the complexes formed.

Stability constant determination

In solution, the ability of calixarenes to complex nonionic molecules is usually determined by NMR spectroscopy (6,28). Nevertheless, it has been demonstrated that LC with cyclodextrins or calixarenes mobile phases could be used to evaluate the stoichiometry and all relevant binding constants for most host–guest systems. RP systems containing calixarenes in the mobile phase solutions may involve many species of the solute (neutral, ionic, free, or bound to one or more calixarene molecules). Supposing that only the neutral molecule is present in the solution and takes part in the process of adsorption and complexation, 1:1 stoichiometry complexes are exclusively formed, and calixarene does not influence the properties of the stationary phase. In the chromatographic column containing solute (*S*) and calixarene (CA), the following equilibria may be established between the mobile phase (*m*) and the stationary phase (*s*):

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

The distribution constant of the solute (K_S), dissociation constant of the complex CA – *S* (K_D), and the distribution constant (K_C) of CA – *S* are given as:

$$K_S = \frac{[S_S]}{[S_m]} \qquad \text{Eq. 1}$$

$$K_D = \frac{[CA_m][S_m]}{[(CA-S)_m]} \qquad \text{Eq. 2}$$

$$K_C = \frac{[(CA-S)_S]}{[(CA-S)_m]} \qquad \text{Eq. 3}$$

In the proposed scheme, the distribution of CA between the phases may be negligible. Because of saturation of the column with CA, the distribution equilibrium of the CA – *S* complex onto the stationary phase may also be neglected (the sorption of this complex must be similar to the sorption of CA itself). The solute capacity factor can therefore be written as:

$$k' = \phi \frac{[S_S]}{([S_m] + [(CA-S)_m])} \qquad \text{Eq. 4}$$

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

$$[CA_T] = [CA_m] + [(CA-S)_m] \qquad \text{Eq. 5}$$

Therefore equation 4 may be expressed as:

$$k' = \phi \frac{K_s K_D}{K_D + [CA_T] - [(CA-S)_m])} \qquad \text{Eq. 6}$$

When the solute concentration is very small compared with the calixarene concentration:

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

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:

$$\frac{1}{k'} = \frac{1}{k_0} + \frac{[CA_T]}{K_D k'_0} \qquad \text{Eq. 8}$$

In cases where two or more calixarenes bind to a solute, traditional equations give curves of increasing slope rather than the straight lines predicted by theory. However, appropriate expressions which take into account multiple CA complexation are easily formulated (20). For a 1:2 (substrate:calixarene) the capacity factor is dependant on the square of the calixarene concentration and equilibrium constants (K, K_1, and K_2) as follow:

$$\frac{1}{k'} = \frac{1}{1/K[A]\phi} + \frac{K_1[CA]}{K[A]\phi} + \frac{K_1K_2[CA]^2}{K[A]\phi} \qquad \text{Eq. 9}$$

In this study, retention data for several substances were measured in the range of concentrations from 0 to 4.10^{-5} mol/L (3.10^{-5} for *p-tert*-butylcalix[8]arene) with *p-tert*-butyldihomooxocalix[4]arene and *p-tert*-butylcalix[4–12]arenes as mobile phase modifiers. For each host–guest system, three measurements were performed, and only the average value was given.

In the case that the ring was smaller than eight phenol units, we did not observe any decrease of the t_R when calixarene's concentration was increased. We can conclude, then, that no complexation took place here. Parts of organic components of the mobile phase are possibly included in cavities of the calixarenes. Such inclusion complexes are described for several organic solvents (6, 29–31) and may also influence the inclusion of solutes in those cavities. Furthermore, Gutsche and Alam (3) described association constants of a lot of PAHs with calix[6] and [8]arenes that are higher than those with smaller calixarenes. A deeper inclusion of large PAHs in the hydrophobic cavities of calix[8] and *p-tert*-butyl-calix[8]arenes is more likely because of the higher conformational flexibility of these hosts. That is, different from a conformational rigid calix[4]arene.

For large calixarenes, capacity factors recorded are summarized in Tables IV and V. When $1/k'$ versus calixarene concentration exhibit a correlation coefficient (*r*) greater than 0.9 (Table IV), representative plots are given (Figure 2). The straight linear relationship confirms formation of the complexes in the mobile phase with a 1:1 stoichiometry, and then stability constants have been calculated from equation 8 ($K_A = K_D^{-1}$) (Table VI). Although, it should always be borne in mind that the lack of generally accepted substance or method or both for precise measurement of t_o in RP–HPLC may cause some errors in the

Table VI. Stability Constants of *p-tert*-Butylcalix [8–12]arenes Complexes with Organic Substances*

Substance	Calixarene	KA(M^{-1})
Anthracene	10+	781
	12+	1468
Naphthalene	9+	1296
	12+	2648
Thianaphthene	11+	1817
m-Xylene	8+	3606
	10+	1910
	11+	2269
	12+	2725
p-tert-Butylphenol	8+	9338
Pentachlorophenol	8+	7672
	9+	2007
	11+	2776
Fluoranthene	9+	1226
	11+	1816
Dibenzothiophene	12+	1540

measurements of k' values, especially when they are small. As shown in Table VI, the calculated stability constants of the complexes vary in the range of 781 – 9338M^{-1}, depending on the number of phenol units of the host and the nature of the aromatic guest molecules. The highest stability constants were obtained for *p-tert*-butylphenol (9338M^{-1} with calix[8]arene) and the lowest for anthracene (781M^{-1} with calix[10]arene). These results show stronger complexation than literature data indicated (using the same method) whenever cyclophane is used (calixarenes, resorcinarenes, or cyclodextrins). In both first cases, the macrocycle owns a more or less rigid conformation [cone (25) or crown–boat conformation (24,27)]. With cyclodextrins, in spite of their having a similar cavity-shaped architecture, a major difference with calixarenes exists in the conformational freedom. The rotation of each phenol unit still remains in the calixarene cavity, whereas the cyclodextrin cavity is conformationally fixed. A free conformation allows a good fit between host and guest, which is one of the factors of a strong complex.

The *p-tert*-butylcalix[8]arene as the mobile phase additive leads to decreased t_R and capacity factors of all guest molecules (Tables IV and V). Furthermore, in cases in which the relation-

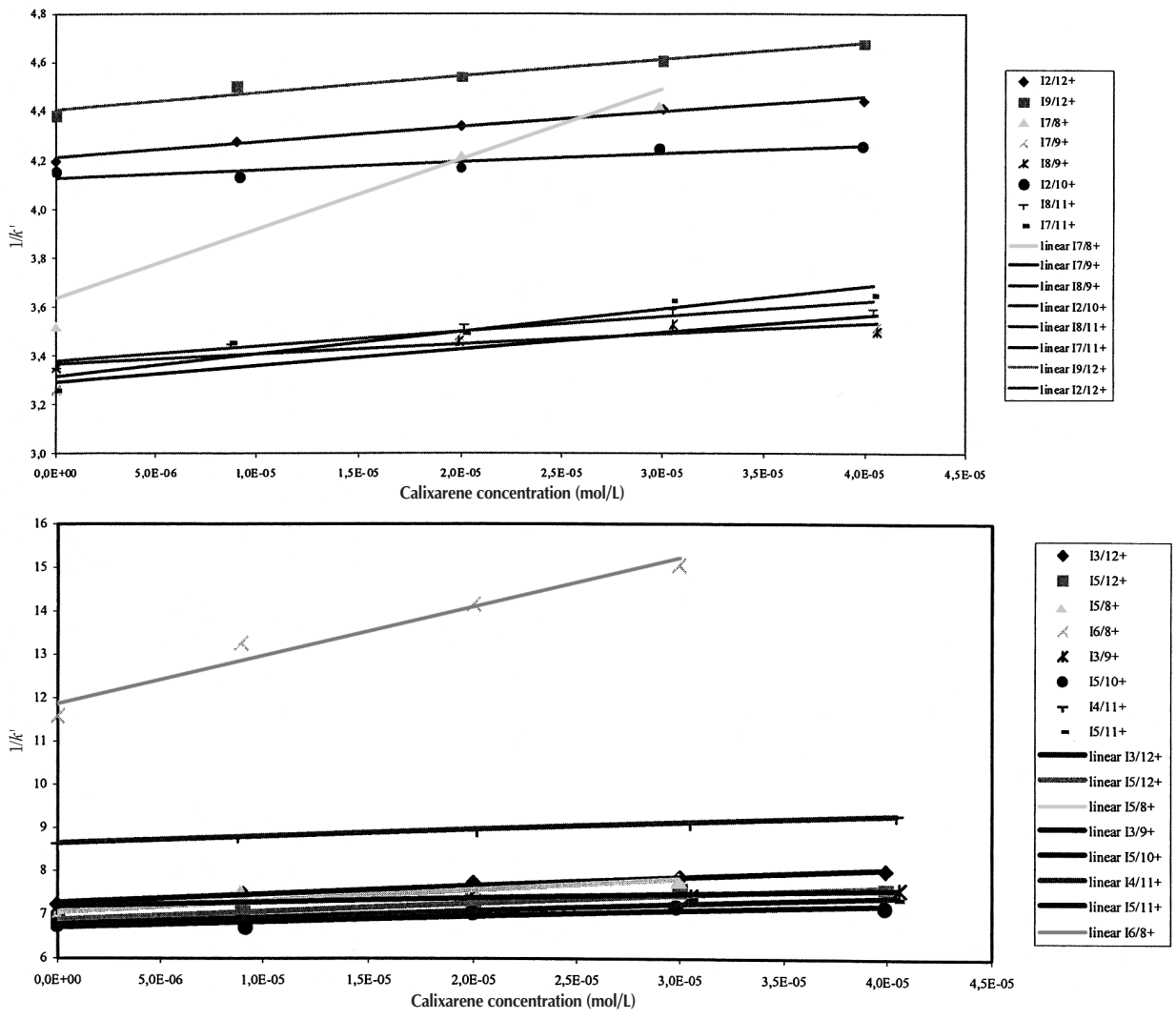

Figure 2. Plots of $1/k'$ versus calixarene concentration when $1/k' = f([calix])$ and the correlation coefficients are greater than 0.9.

ship between $1/k'$ and (CA) was linear, calculated association constants (Table VI) exhibit the greatest values (3606–9338M^{-1}). Nevertheless, it is important to consider that experiments were only performed for the first three concentrations (because of the lack of solubility). The good ability to form an inclusion complex of this calixarene can be explained in terms of conformation. The solid-state structure of *p-tert*-butylcalix[8]arene is known from an *X*-study (32) and consists of "pleated loops," with the OH groups engaged in circular hydrogen bonding. The ^{1}H NMR data are best interpreted in terms of this solid-state conformation, which is supposed to be present also in solution (5). However, the number of possible conformers in solution is much larger, and if one considers only rigid up-and-down orientations of the phenyl groups relative to the mean plane of O atoms, there are 16 distinct conformers (5). Their numbers increase if this restriction is relaxed and the phenyl groups are allowed to orient in a more flexible manner.

Conformation of large calixarenes has not been extensively studied. Indeed, Stewart and Gutsche (16) have demonstrated that the barriers to conformational interconversion generally trend downward as the size and concomitant flexibility of the calixarene increase. The ability to include aromatics guests of *p-tert*-butylcalix[8–12]arenes may derive from their good flexibility in solution, and the large size of the cavity permits it to bind to both the solvent's molecules and guest solute.

Capacity factors represented Table V seem to be influenced by adding calixarene in the mobile phase. But determined k' values don't satisfy the linear relationship $1/k'$ versus (CA) from equation 8. It means that an assumption of 1:1 stoichiometry of the calixarenes complex is not valid for this system. We suggested, then, the possibility of a 2:1 calixarene–guest complex (equation 9), but the capacity factor is not dependant on the square of calixarene's concentration in a satisfactory manner. To summarize, a possible complexation between calixarenes present in the mobile phase and the aromatics guests can be envisaged, but more experiments are necessary to prove it. Indeed, we observed a decrease in the t_R as (CA) increased (no equation allowed us to model our experimental data). To our knowledge, such an HPLC complexation study has not even been realized using a four-components mobile phase; we can easily suppose that each of the components influences the analysis conditions and final result. Not only can each molecule of solvent be complexed itself by the calixarene, but also each component fights with calixarene for the place on the surface of support of the chromatographic column. From this point, the lack of reproducibility of the measurements can be explained.

Conclusion

The *p-tert*-butylcalix[8–12]arenes are able to form host–guest inclusion complexes with several aromatic component systems. With such hosts, RP–HPLC is a useful tool in preliminary complexation studies. We have been able to compare a large family of *p-tert*-butylcalixarenes and confirm that calix[8]arene is a good receptor.

Work is currently being undertaken to functionalize calix[8]arene to improve its complexation properties towards nonionic molecules.

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