Analysis of the p-*tert*-butylcalix[4]arene bis-crown Derivative (Dc3)-Acetonitrile Host−Guest Complexing Behavior by Nuclear Magnetic Resonance (NMR) Spectroscopy and Computational Methods

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We report the analysis of the structure and dynamics of the calix[4]arene bis-crown derivative (Dc3) and the host−guest system formed by Dc3 and acetonitrile in a chloroform solution. NMR spectral measurements, molecular mechanics, and molecular dynamics calculations have been used. Results showed that the host−guest complex in solution has chemical shift values that are considerably different from those of the pure compounds and from the Dc3-acetonitrile mixture. Molecular mechanics simulations showed that Dc3 has a cavity with an almost circular cone shape and that the CN group of acetonitrile in the host−guest complex pointed toward the outside of the cavity. This orientation appeared energetically favored. The value of the generalized order parameter (S^2) from ^{13}C-NMR relaxation rates also showed that the methyl group of acetonitrile behaved as a free rotor in the Dc3 cavity with reorientational motion of almost two orders of magnitude faster than the aromatic carbons.

Introduction

The interest in synthetic macrocycles calix[*n*]arenes arises from their ability to form inclusion complexes with ions and small organic molecules. Calix[n]arenes have a wide range of applications as catalysts, ion carriers, and model structures for biomimetic research.[1,2] Calix[4]arenes are macrocyclic tetramers synthesized from the condensation of phenol and formaldehyde. These compounds can be easily functionalized, making them attractive starting materials for the preparation of specific hosts with tailor-made properties.[3−5] In the past decade, calix[*n*]arenes have attracted much attention as versatile host molecules, because of the ready availability of a wide range of calix[*n*]-arenes and the simplicity in the chemical modifications at the lower and/or upper rim.[6−8] Previous studies on the synthesis and conformational of calixarene derivatives in bulk phases have been performed, mainly using NMR techniques and X-ray analysis.[9−11] A detailed investigation on the conformational properties of calix[*n*]arenes and their inclusion compounds with ions, small organic molecules, and fullerenes at the interface of several dispersed systems (planar monolayers and curved interfaces such as micelles) has been previously reported by some of the authors of the present paper.[12−18]

Calix[4]arenes may adopt four different conformations: cone, partial cone (paco), 1,2-alternate (1,2-alt), and 1,3-alternate (1,3-alt). Depending on the substituents, these conformations can be flexible or rigid on the laboratory time scale.[19−21]

Calix[4]arene derivatives adopt the cone conformation when stabilized by a cyclic array of intramolecular hydrogen bonds.[22−24] The endo-OH groups can pass through the center of the macrocycle, and this cone-to-cone interconversion (topomerization) can be easily monitored by variable-temperature ^{1}H NMR, because methylene protons are exchanged.[21] Under slow exchange conditions, on the NMR time scale, methylene protons show a pair of doublets that broaden and coalesce into a singlet when the temperature is increased. The energy barrier of aryl rotation through a symmetric calix[4]arenes annulus with a 1,3-alt conformation has been found for many calix[4]arenes compounds.

The inclusion of small organic guests (acetonitrile, alcohols) by calix[4]arene, studied by ^{1}H- and ^{13}C-NMR spectroscopy,[25−28] has been recently reported. The guest is included into the host hydrophobic cavity through the apolar aliphatic residues of the latter.[29,30] The inclusion process is controlled by subtle conformational and electrostatic effects.[31,32]

NMR relaxation experiments (T_1, nuclear Overhauser effect (NOE)) are very important tools to study the internal dynamics of biomolecules. Molecular information can be extracted from the relaxation data using various analytical models for the dynamics, whose parameters are derived from the fit of relaxation data.

The most commonly used approach is based on the so-called "model-free" formalism by Lipari and Szabo.[33,34] The informa-

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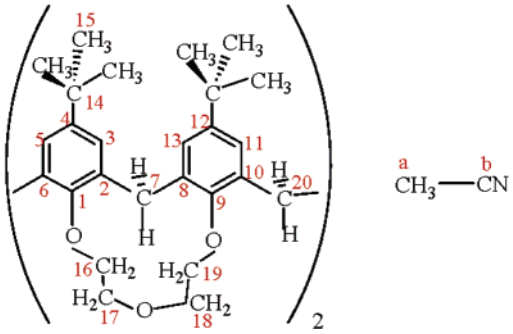

Figure 1. Structure and numbering of Dc3 and acetonitrile.

tion contained in relaxation data is assumed to be fully described by two quantities: a generalized order parameter (S^2), which is a measure of the spatial restriction of the internal motion, and an effective correlation time (τ_e), which is a measure of the internal motions.

In this study, the dynamic and conformational of a calix[4]-arene, modified by insertion of a pair of crown ether units, on the lower rim (Dc3) have been evaluated by NMR studies, as well as molecular mechanic and dynamic calculations. In particular, the properties of the Dc3 in a chloroform solution and in the presence of guest (i.e., acetonitrile) have been analyzed.

Experimental Section

The structure and numbering of p-*tert*-butylcalix[4]arene bis-crown-*m* studied in this paper is shown in Figure 1. This compound is identified by the Dc*m* acronym, where *m* is the number of O atoms in the etheric chain that connects the two aromatic moieties.

The Dc3 and Dc3−CH_3CN complex were synthesized as reported in a previous paper.[35] Solutions of Dc3 and the Dc3−CH_3CN complex (∼5 mM) were prepared in $CDCl_3$.

The systems investigated in this paper are (i) Dc3 in $CDCl_3$ solution; (ii) Dc3−CH_3CN complex in $CDCl_3$ solution, henceforth indicated as the Dc3-acetonitrile host−guest complex; and (iii) a mixture of Dc3 and CH_3CN in $CDCl_3$ solution, obtained by mixing Dc3 and CH_3CN in 1:1 ratio. (This last mixture will be indicated as "the mixture of Dc3-acetonitrile".)

NMR Methods. NMR experiments were performed with a Bruker model DRX-600 AVANCE spectrometer that was equipped with an *xyz*-gradient unit, operating at 600.13 and 150.89 MHz for ^1H and ^{13}C, respectively. A reverse triple resonance (^1H, ^{13}C, BB) probe head with *xyz*-gradients for two-dimensional (2D) experiments, and a double resonance (^1H, BB) probe with *z*-gradients was used for ^{13}C inversion recovery experiments.

^{13}C spin−lattice relaxation rates were measured using the inversion recovery method, which included a gate decoupling pulse sequence. Data analysis was performed by a three-parameter exponential regression of the longitudinal magnetization recovery curves. The variable delay assumed the following values: $\tau = 0.05$, 0.01, 0.02, 0.04, 0.08, 0.1, 0.2, 0.4, 0.8, 1.0, 5.0, 8.0, 15.0, and 30 s with a recycle time of $d_1 = 30$ s. The maximum experimental error was 5% for R_1.

The Levenberg−Marquardt algorithm was used for nonlinear curve fits for all experimental relaxation decay data.

The longitudinal ^{13}C spin−lattice relaxation times of all protonated carbons were measured in $CDCl_3$ solutions at temperatures in the range of 240−308 K.

NOESY,[36] dqf-COSY, and HMQC[37] were acquired with 2048 complex points for 256 experiments with a recycle delay of 8 s and a TPPI phase cycle. A 45° shifted squared-sine window function was applied in both dimensions for each set of data and zero-filled to 1024 points along F1. NOESY sets of spectra were acquired with mixing times of 250 and 800 ms, respectively.

All the experiments were performed with a spectral width of 8.0 ppm and a transmitter offset of 4.7 ppm.

Data were processed with the NMRpipe[38] (version 3.3) software, and 2D spectra were analyzed with the SPARKY software.[39]

Molecular Modeling and Structure Refinement. Molecular dynamic simulations were performed with the MACROMODEL 5.0 software,[40] implemented on INDIGO 2 Solid Impact and Octane computers (Silicon Graphics, SGI) that were equipped with RISC R10000 and R12000 processors, respectively, working under the IRIX 6.3 and the IRIX 6.5 operating systems. The energy minimization procedure was performed with the MM2 force field.[41]

The molecular model of Dc3 with $CDCl_3$ solvent was built by the MACROMODEL software. Periodic boundary conditions were applied for the entire simulation. The integration step size of 1 fs SHAKE algorithm[42] was applied. The molecular dynamic simulations present sequential variations of the temperature. The starting structure was subjected to (i) an equilibration period of 10 ps at 10 K, (ii) heating to 300 K in 50 ps, (iii) holding at 300 K for 50 ps, (iv) heating at 700 K in 60 ps, (v) holding at 700 K for 50 ps, (vi) cooling at 300 K in 30 ps, and (vii) cooling at 10 K in 10 ps. Experimental constraints were applied to all the dynamics simulations. The system obtained at the end of the simulation was subjected to energy minimization without constraints.

TABLE 1: Chemical Shift Values of ^1H for Dc3 and the Dc3-Acetonitrile Complex in $CDCl_3$

proton	δ (ppm)
H(3/13)	7.07
H(5/11)	7.06
H(7endo)	5.02
H(20endo)	4.47
H(17/18)	4.27
H(16′/19′)	4.25
H(16″/19″)	3.92
H(20exo)	3.25
H(7exo)	3.21
H(15)	1.20
H(a)[a]	−0.57

[a] Proton atom of the acetonitrile molecule.

Results and Discussion

Assignment Analysis of Dc3 and the Dc3-Acetonitrile Host−Guest Complex. The first step of this study was the definition of both the proton and carbon NMR spectral properties of the two compounds. In Table 1, the proton NMR chemical shift of Dc3 and the chemical shift of the methyl group in the Dc3-acetonitrile host−guest complex are reported. The proton assignment was obtained using dqf-COSY (see Supporting Information) and NOESY experiments. The aromatic H(3,5-11,13) protons were assigned using chemical shift considerations and on the basis of the NOESY spectrum, which showed dipolar couplings between the H(7exo) and H(3/13) and H(20exo) and the H(5/11) protons. These dipolar couplings also could be observed in the host−guest Dc3-acetonitrile NOESY spectrum (see Figure 2). This allowed the unambiguous identification of the H(3/13) protons (7.07 ppm) and the H(5/11) protons (7.06 ppm). The NOESY spectrum was also used to assign the four

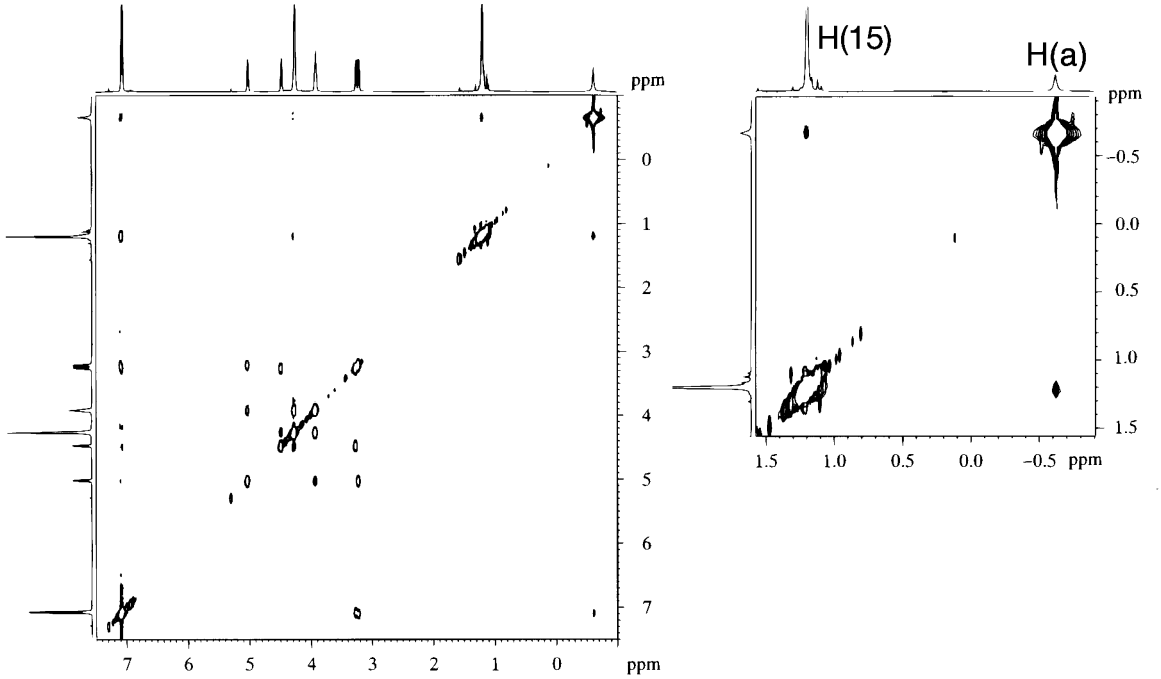

Figure 2. NOESY spectrum of the Dc3-acetonitrile host−guest complex recorded at 600 MHz and 298 K. Inset reports the detail of the cross-peak between the H(a) of acetonitrile and H(15).

equivalent H(17) and H(18) methylene protons on the basis of H(17/18)−H(16/19) dipolar couplings. The signals observed at 4.25 and 3.92 ppm were assigned to the H(16/19) diasterotopic protons; however, their identification is not discussed in this paper. The signal of the six equivalent methyl groups of Dc3 was observed at 1.20 ppm in the proton spectrum. On the basis of the spectral assignment of Dc3, we defined the Dc3 conformation in solution. The presence of one pair of doublets for the methylene bridge atoms, H(7) and H(20), suggested that the molecule assumed a cone structure. This comes from the following findings: the methylene bridge showed an AB-type scalar coupling, H(7exo) at 3.21 ppm with $J = 12.2$ Hz and H(7endo) at 5.02 ppm with $J = 12.2$ Hz and H(20exo) at 3.25 ppm with $J = 12.0$ Hz and H(20endo) at 4.47 ppm with $J = 12.0$ Hz, which are the same as those ascribed to the calixarene cone form, with an apparent C_4 symmetry.[43,44] This structure is the energetically favored conformation.

Structural Analysis of Dc3 and Dc3-Acetonitrile Host−Guest Complex. The Dc3-acetonitrile host−guest complex and Dc3 in solution showed very similar ^{1}H spectra. The acetonitrile proton signal occurred at −0.57 ppm, i.e., a shift of 1.64 ppm toward higher field, with respect to the pure acetonitrile resonance.[45] The observed upfield shift is attributed to the host−guest complex formation, which induced a strong shielding contribution, generated by the four aromatic rings around the acetonitrile methyl protons.

A similar behavior was observed in the carbon chemical shifts for both Dc3 and the Dc3-acetonitrile complex. Table 2 reports the carbon chemical shift values of the host−guest complex obtained by hetero-correlation 2D spectrum (Supporting Information available). Similar to the proton chemical shift of the acetonitrile methyl group, the carbon signal of the same group showed an upfield shift to −3.00 ppm, i.e., a shift of 4.30 ppm toward higher field, with respect to the pure acetonitrile.[46]

A mixture of Dc3-acetonitrile (1:1) in $CDCl_3$ was then prepared and investigated. Both proton and carbon spectra resulted as the sum of the pure components. To verify the motion conditions of Dc3 and the Dc3-acetonitrile host−guest complex, additional carbon relaxation rate measurements (R_{1exp}), as a function of temperature (253, 247, and 240 K), have been performed. These experimental R_{1exp} values versus $1/T$ indicate that the extreme narrowing conditions are not fulfilled at low temperature. The Lipari−Szabo approach was applied and the S^2 values calculated for the Dc3 and Dc3-acetonitrile host−guest complex (see below).

TABLE 2: Chemical Shift Values of ^{13}C for Dc3 and Dc3-Acetonitrile Complex in $CDCl_3$

carbon	δ (ppm)
C(5/11)	126.17
C(3/13)	125.12
C(b)N[a]	117.63
C(16/19)	76.62
C(17/18)	75.25
C(14)	34.46
C(15)	31.80
C(7)	31.39
C(20)	30.43
C(a)H_3[a]	−3.00

[a] Carbon atom of the acetonitrile molecule.

No chemical shift variations were detected for the acetonitrile signals, with respect to the pure compound.

These findings supported the following considerations:

(1) Acetonitrile can be hosted inside the Dc3 cavity, and the host−guest complex is stable when dissolved in $CDCl_3$;

(2) The methyl group resonance is the only signal affected by the complex formation, suggesting that this is the group most involved in the interaction; and

(3) The Dc3-acetonitrile (1:1) mixture did not show any change in the spectrum, with respect to the pure compounds.

We can argue that even if the complex is thermodynamically favored, its formation is kinetically excluded. This is also supported by the fact that the host−guest complex is stable in $CDCl_3$ solution and does not show any tendency to dissociate.

The information on the host−guest interaction obtained by both proton and carbon spectra were then confirmed by studying the through-space intramolecular dipolar connectivities observed in the NOESY spectrum. NOESY spectra of Dc3 and of the host−guest complex showed similar intramolecular cross-peak

TABLE 3: Dipolar Correlation for Dc3-Acetonitrile Host−Guest Complex in Solution Obtained from the NOESY Spectrum

Cross-peak		
strong (1.8−2.5 Å)	medium (2.5−3.7 Å)	weak (3.7−4.3 Å)
H(7exo)−H(3/13)	H(7endo)−H(16/19)	H(7exo)−H(15)
H(20exo)−H(5/11)	H(20endo)−H(17/18)	H(20exo)−H(15)
H(15)−H(5/11)	H(a)−H(3/13)	H(a)−H(15)
H(15)−H(3/13)	H(a)−H(5/11)	

patterns. Dipolar connectivities in the form of cross-peaks from intramolecular interactions were detected. These cross-peaks included (i) H(7exo)−H(3/13) and H(20exo)−H(5/11), (ii) H(7endo)−H(16/19) and H(20endo)−H(17/18), (iii) H(15)−H(3/13) and H(15)−H(5/11), (iv) H(15)−H(7exo), and (v) H(15)−H(20exo). In addition to intramolecular cross-peaks, three specific intermolecular interactions were detected in the host−guest system. These interactions involved the acetonitrile methyl protons (H(a)) and several groups of the calixarene molecule. These cross-peaks from intermolecular Dc3-acetonitrile interactions included (vi) H(a)−H(3/13), (vii) H(a)−H(5/11), and (viii) H(a)−H(15).

NOESY constraints were classified using the cross-peak volume.[47,48] This semiquantitative method allows us to define three categories of constraints: strong (1.8−2.5 Å), medium (2.5−3.7 Å), and weak (3.7−4.3 Å). The dipolar correlations for the host−guest complex are reported in Table 3.

From the observed dipolar connectivities between the acetonitrile methyl protons and the aromatic protons of Dc3, as well as between the H(a) and Dc3 methyl (H(15)), conclusive evidence of the host−guest interaction has been obtained. These results also suggested that the acetonitrile methyl group was included inside the Dc3 cavity, whereas the cyano group seemed to be located in an external position outside of the cavity. This hypothesis is based on the chemical shift changes that involved the carbon cyano group chemical shift of acetonitrile in the host−guest and in the pure compound. Similarly to Dc3 in solution, X-ray studies on the host−guest crystal[35] reports that the location of the cyano group is outside the Dc3 cavity.

To examine the conformation of the host−guest complex, experimental constraints derived by the NOESY spectrum have been used in molecular mechanic calculations. Molecular mechanic calculations using the MM2 force field were performed, to provide insights into the energetic stability of the complex. The stationary points along the reaction coordinates and the minima, obtained from the stochastic search, were refined using the full matrix Newton−Raphson minimization algorithm.[49,50] During the calculation, an energy minimum was observed for the acetonitrile molecule located inside the Dc3 cavity with the nitril group outside. This conformation was then used as a starting point for molecular dynamic simulations. The temperature profile of this simulation is reported in the Experimental Section. The structure obtained is shown in Figure 3. This result further supports the hypothesis of a cone structure for the Dc3 system. The acetonitrile guest is located at the top of the Dc3 cavity with the methyl group inserted inside the cone and the CN group pointing outward.

Dynamical Analysis of the Dc3-Acetonitrile Host−Guest Complex. Reorientations of the entire molecule, or of molecular segments, are studied by relaxation data analysis. The overall and segmental mobility of both Dc3 and the Dc3-acetonitrile host−guest complex were studied by measurement of their ^{13}C spin−lattice relaxation time (T_1).

The relaxation of ^{13}C nuclei in medium-sized molecules is generally determined by dipolar interactions with directly bound

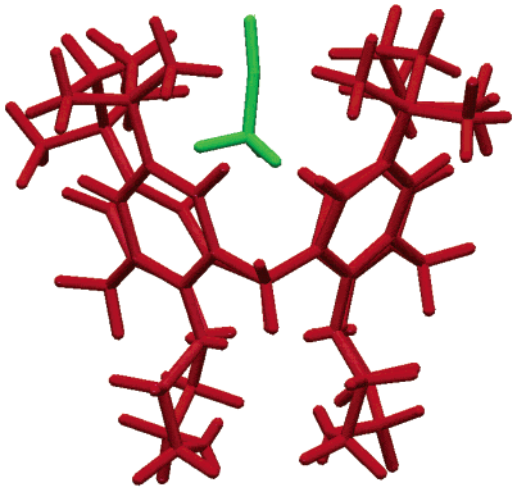

Figure 3. Conformational structure for the Dc3-acetonitrile host−guest complex obtained by a combined analysis of NMR spectra and molecular dynamic simulations (side view, cone conformation).

protons. When the relaxation times are measured under ^1H decoupling conditions, the cross-relaxation term vanishes. The intramolecular dipolar longitudinal relaxation rate for the *i*-th ^{13}C nucleus by its n_H bound protons is related to the molecular reorientations by[33,34]

$$\left(\frac{1}{T_1}\right) = n_H \frac{\hbar^2 \gamma_C^2 \gamma_H^2}{10 r_{CH}^6} [J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)] \quad (1)$$

where γ_C and γ_H are the magnetogyric ratios of the ^{13}C and ^1H nuclei, respectively; r_{CH} is the length of the internuclear vector between the carbon and proton atoms (1.08 Å);[51,52] and $J(\omega)$ are the spectral densities, which are dependent on the resonance frequencies of the ^{13}C and ^1H nuclei (ω_C and ω_H, respectively).

The NOE factor of the *i*-th C atom is given by

$$\mathrm{NOE} = \frac{\gamma_H}{\gamma_C} \frac{\sum_{j=1}^{n_H} \sigma_{ij}}{\sum_{j=1}^{n_H} \rho_{ij} + \rho_i^*} \quad (2)$$

where σ_{ij} is the cross-relaxation rate, ρ_{ij} the dipolar relaxation rate, and ρ_i^* the so-called leakage term, which represents the contribution of all the relaxation mechanisms other than dipole−dipole interaction. Under ^1H decoupling conditions, the sum of ρ_{ij} over all the n_H interacting protons gives the dipolar spin−lattice relaxation rate. For ^{13}C relaxation via intramolecular dipolar interaction, $\rho_i^* = 0$, and the NOE factor reaches its maximum value, which is dependent only on the reorientational molecular dynamics:

$$\mathrm{NOE} = \frac{\gamma_H}{\gamma_C} \frac{6J(\omega_H + \omega_C) - J(\omega_H - \omega_C)}{J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)} \quad (3)$$

Aromatic ^{13}C atoms relax, even in moderate magnetic fields, partially via the chemical shift anisotropy (CSA) mechanism. The corresponding longitudinal relaxation rate is

$$\left(\frac{1}{T_1}\right)_{CSA} = \frac{1}{15} \gamma_C^2 H_0^2 (\Delta\sigma_i)^2 J(\omega_C) \quad (4)$$

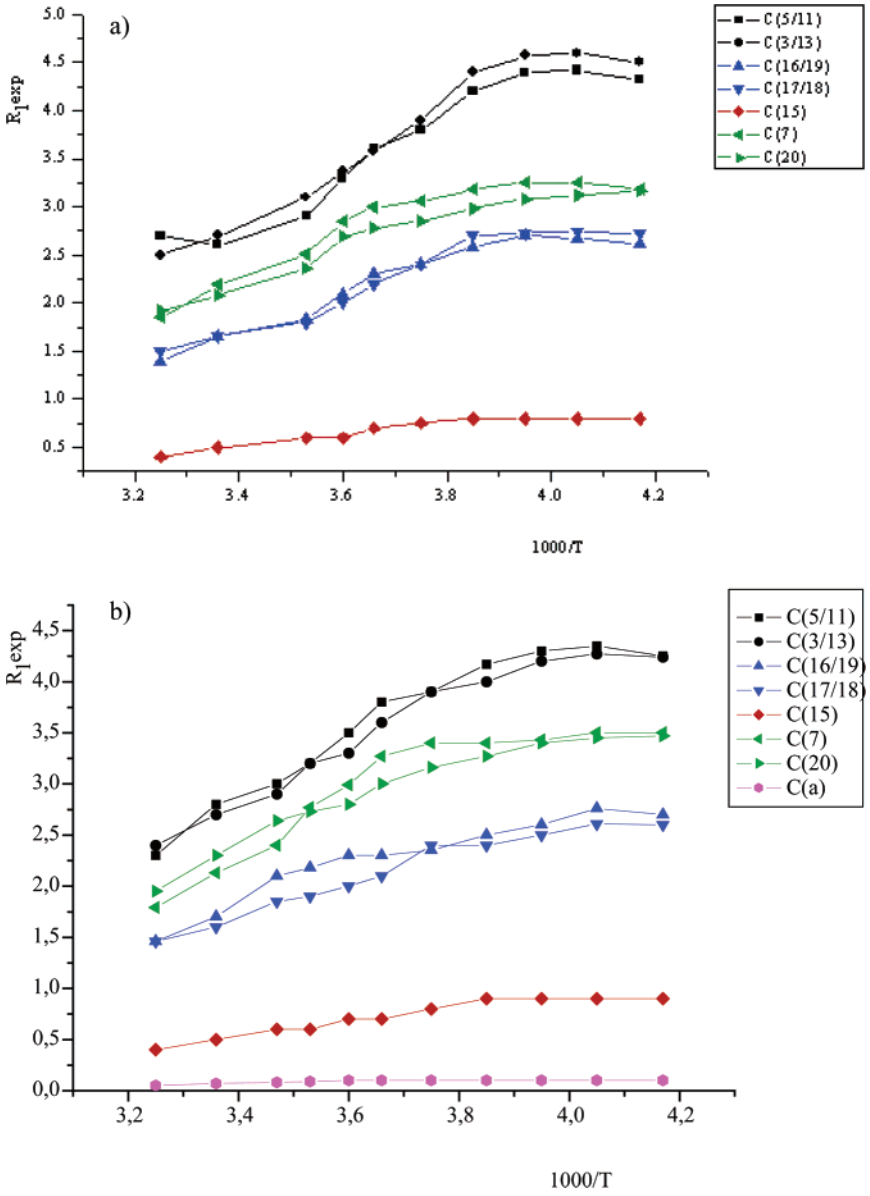

Figure 4. Experimental ^{13}C relaxation rates (R_{1exp}, in units of s^{-1}) versus the reciprocal of temperature (1000/T) for (a) Dc3 and (b) the Dc3-acetonitrile host−guest complex. Error was estimated as the maximum value of 5%.

where H_0 is the magnetic field strength and $\Delta\sigma = \sigma_{||} - \sigma_{\perp}$ is the CSA for an axially symmetric chemical shift tensor. $\sigma_{||}$ and $\sigma_{\perp}$ are the respective parallel and perpendicular components, with respect to the principal axis of the chemical shift tensor. It is reasonable to assume that the aliphatic methylene and methynic ^{13}C nuclei of calix[4]arenes relax exclusively via the dipolar relaxation mechanism.[4,53]

The spectral densities are related to the effective correlation time (τ_c) for the reorientation of the corresponding internuclear $^{13}C-^{1}H$ vectors by[54]

$$J(\omega) = \frac{2\tau_c}{1 + (\omega\tau_c)^2} \tag{5}$$

with $\tau_c^{-1} = \tau_e^{-1} + \tau_m^{-1}$, where τ_m is the correlation time for the overall tumbling of the molecule and τ_e is the effective internal correlation time.

In the extreme narrowing regime, when the product of the molecular reorientational correlation time and the resonance frequency is much less than unity ($\omega\tau_c \ll 1$), eq 5 reduces to

$$J(\omega) = 2\tau_c \tag{6}$$

The temperature dependence of any correlation time is described by an Arrhenius-type equation:[55]

$$\tau_c = \tau_0 \exp\left(\frac{E_a}{RT}\right) \tag{7}$$

where E_a is the activation energy for the corresponding molecular motion. To take into account very fast internal motions, the "model-free" approach by Lipari and Szabo has often been used.[33,34] It reduces the spectral density by a factor S^2, which is the generalized order parameter:

$$J(\omega) = S^2\left(\frac{2\tau_c}{1 + (\omega\tau_c)^2}\right) \qquad (\text{for } 0 \le S^2 \le 1) \tag{8}$$

The observed relaxation rates ($R_{1exp} = 1/T_1$) are shown in Figure 4a and b as a function of $1/T$ for the Dc3 and Dc3-

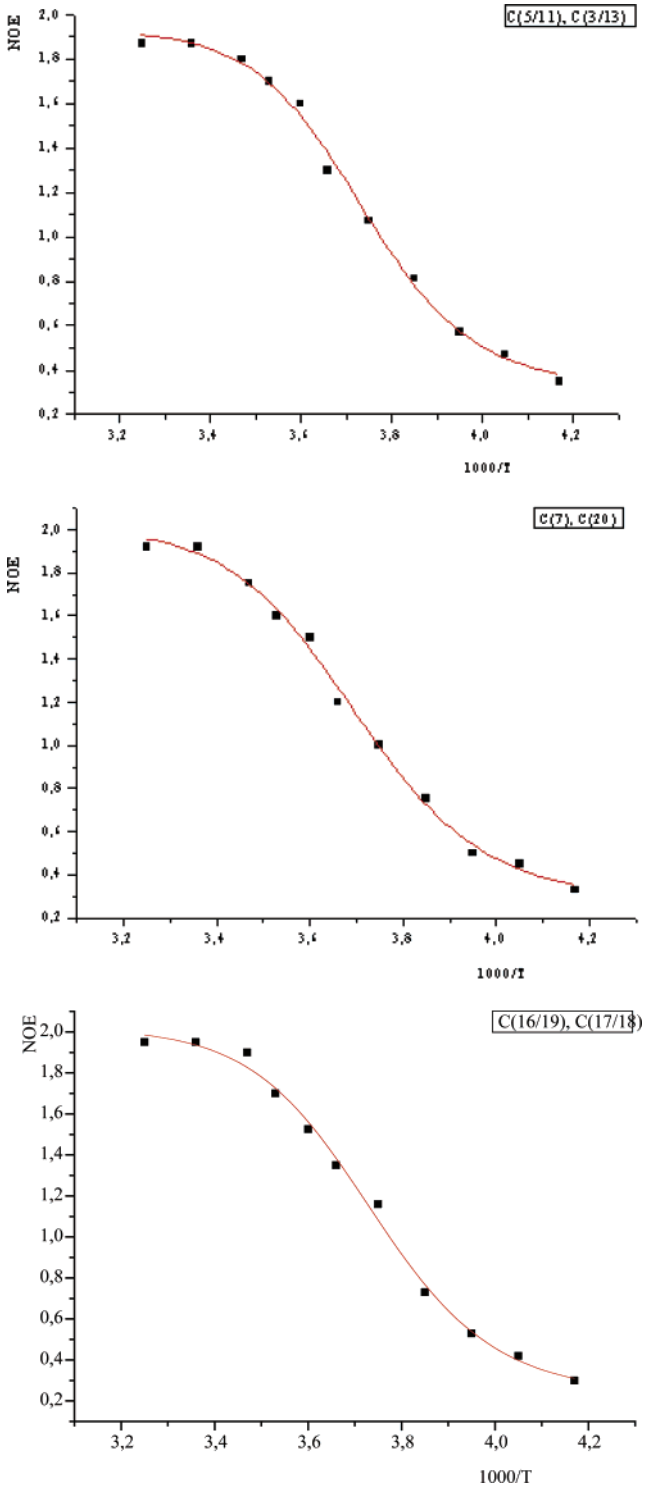

Figure 5. Experimental and calculated NOE values as a function of reciprocal temperature (1000/*T*) for the aromatic carbons C(5/11) and C(3/13) and the methylenic carbons C(7), C(20), C(16/19), and C(17/18) in the Dc3-acetonitrile host−guest complex. Error was estimated as the maximum value of 7%.

acetonitrile host−guest complex, respectively. The carbon relaxation rate exhibits a maximum value at low temperature for all systems. The measured $\{^1H\}-^{13}C$ NOE values are plotted as a function of reciprocal temperature $1/T$ in Figure 5a−c. As shown in the figures, the NOE factor decreases as the reciprocal temperature increases.

The observed ^{13}C relaxation rates have been interpreted in terms of molecular motions, and the experimental NOE factors have been compared with the theoretical values expected for carbon nuclei totally relaxing through $^1H-^{13}C$ dipolar interactions. In our case, for the methyl groups C(15), which are expected to satisfy the fast motion conditions, the theoretical maximum value calculated for the NOE is equal to 1.98. The theoretical maximum NOE factor for the aromatic carbons C(5/11) and C(3/13), for the methylenic carbons C(7) and C(20), and for the methylenic carbons of the etheric chain C(16/19) and C(17/18) are equal to 1.87, 1.92, and 1.95, respectively. These NOE_{max} values correspond to a range of correlation times of 0.25 and 0.40 ns. For the DC3 and Dc3 host−guest complex, this correlation time is assumed to be the correlation time for the skeleton motion.

The fractional effectiveness of the dipolar relaxation for all C atoms is given by the ratio $\chi^{DD} = NOE_{exp}/NOE_{theor}$, and it was used to calculate the dipolar spin−lattice relaxation rate R_1^{DD} ($R_1^{DD} = \chi^{DD}R_{1exp}$). R_1^{DD} values, as a function of the reciprocal temperature (see Figure 6a and b). As R_{1exp}, R_1^{DD} reaches its maximum value at low temperature (247 K).

To investigate the reorientational dynamics of the calix[4]-arenes systems in solution, the experimental longitudinal relaxation rate and NOE factors of the ^{13}C nuclei have been simultaneously fitted using eqs 1, 3, 7, and 8.

The best-fit parameters are listed in Table 4.

The experimental ^{13}C spin−lattice relaxation rate values suggest the following:

(1) The Dc3 in chloroform solution does not experience fast motion conditions at temperatures of <260 K. The same results have been found for the Dc3-acetonitrile host−guest complex.

(2) The methyl group of acetonitrile showed S^2 values smaller than the methyl group of Dc3 and totally independent from the Dc3 reorientation. Even if the molecule is hosted in the Dc3 cavity, its behavior is typical of a free rotor.[56]

Results shown in Table 4, and in Figure 4a and b, suggest that the entire reorientation of Dc3 in both free and complex systems is not fast at low temperature. The comparison of the order parameters, S^2, calculated for the C atoms of the calix-[4]arenes derivatives suggests that the dynamical behavior of Dc3 is not affected by the presence of the guest molecule (CH_3-CN).

The order parameter, which describes the special resolution of fast internal motions, of the methylenic carbons C(16/19) and C(17/18) is smaller, compared to the cone of the calix[4]-arene structure, showing a greater freedom of motion for the etheric chain.

Comparison of the activation energy (E_a) values of the two systems allows the following considerations:

(1) The aromatic moieties (C(5/11) and C(3/13)) do not exhibit appreciable variation during host−guest complexation.

(2) The methylenic carbons of the etheric chain, C(16/19) and C(17/18), in the Dc3-acetonitrile host−guest complex shows lower E_a values than in Dc3. This result suggests a reduction in the rigidity of the etheric methylenic chain, probably because of a larger cone size as a consequence of the host−guest complex formation.

(3) The activation energy of C(7) shows a remarkable increase in the E_a value for Dc3-acetonitrile, with respect to Dc3. This behavior is the result of the acetonitrile inclusion inside the Dc3 cavity, which modified the rigidity of the methylenic groups bridging the four aromatic units.

(4) Practically no effect on the E_a value of the C(15) methyls was observed upon complexation.

In conclusion, the acetonitrile inclusion in the Dc3 cavity induces an enlargement of the Dc3 cone structure, with a stretching of the C(20) and C(7) methylene-aromatic ring bonds.

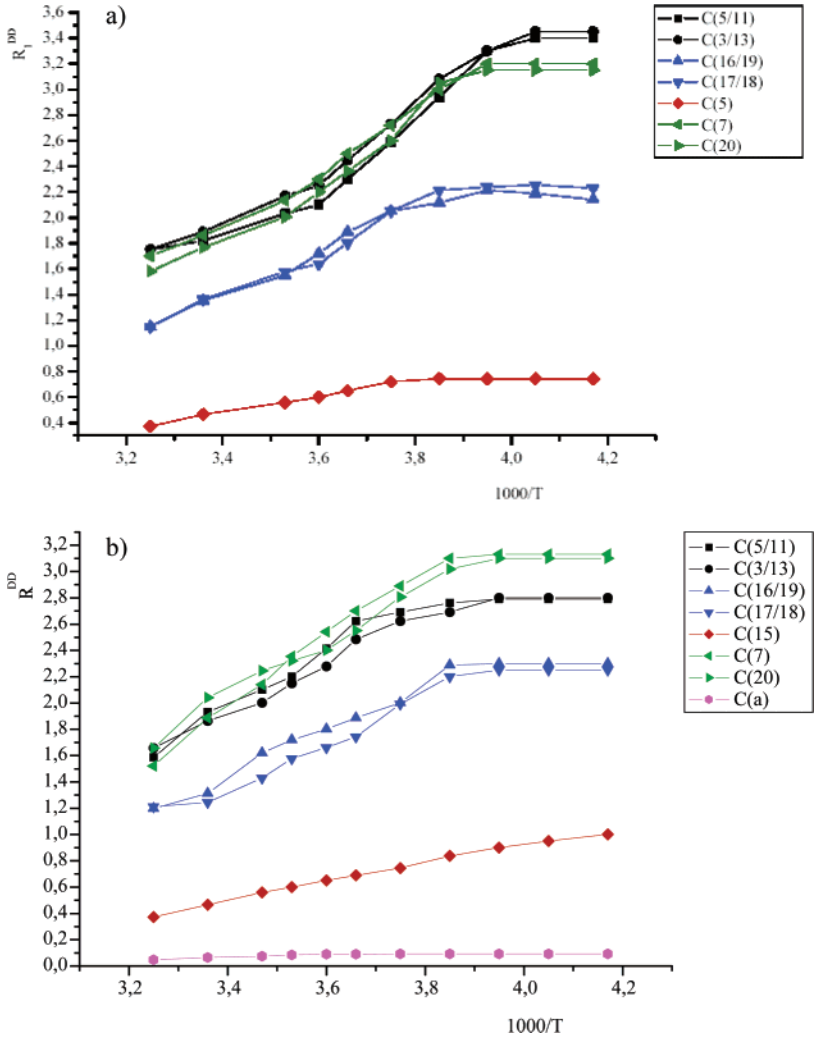

Figure 6. Dipolar ^{13}C relaxation rates (R_1^{DD}, in units of s^{-1}) versus the reciprocal of temperature (1000/*T*) for (a) Dc3 and (b) the Dc3-acetonitrile host−guest complex. Error was estimated as the maximum value of 5%.

TABLE 4: Best-Fit Parameters for the Carbon Relaxation Data of the Dc3 and the Dc3-Acetonitrile Host−Guest Complex ($T = 247$ K)

carbon atom	τ_c (ps)	τ_0 (ps)	E_a (kJ/mol)	S^2
		Dc3		
C(5/11)	700	1.1 ± 0.2	13.2 ± 0.4	0.85 ± 0.01
C(3/13)	900	1.0 ± 0.2	13.8 ± 0.4	0.83 ± 0.01
C(16/19)	150	0.6 ± 0.1	11.3 ± 0.5	0.65 ± 0.02
C(17/18)	160	0.9 ± 0.2	10.6 ± 0.5	0.66 ± 0.02
C(15)	37	1.2 ± 0.1	7.04 ± 0.1	0.095 ± 0.001
C(7)	220	0.8 ± 0.1	11.5 ± 0.3	0.77 ± 0.02
C(20)	215	1.0 ± 0.2	11.0 ± 0.4	0.75 ± 0.01
		Dc3-Acetonitrile		
C(5/11)	750	1.3 ± 0.2	13.0 ± 0.4	0.85 ± 0.01
C(3/13)	650	1.1 ± 0.2	13.1 ± 0.5	0.83 ± 0.01
C(16/19)	165	1.0 ± 0.2	10.5 ± 0.4	0.67 ± 0.02
C(17/18)	150	1.6 ± 0.2	9.32 ± 0.5	0.65 ± 0.02
C(15)	43	1.2 ± 0.1	7.34 ± 0.1	0.096 ± 0.001
C(7)	300	0.2 ± 0.1	15.5 ± 0.2	0.78 ± 0.02
C(20)	250	0.8 ± 0.2	11.8 ± 0.3	0.75 ± 0.01
Ca(CH_3CN)	4.5	0.1 ± 0.2	7.81 ± 0.2	0.0250 ± 0.005

This leads to restricted motion of the methylenic groups bridging the aromatic rings and a decrease of the energy barriers for the reorientational motion of the etheric groups. The evidence of an enlargement of the Dc3 cone structure due to the formation of the host−guest complex was derived from the molecular models obtained after structure refinement.

Conclusions

All the experimental data presented in this paper confirm that the interaction between small molecules and calixarene derivatives in solution involve the cone portion of the calixarene ring. The size of the molecular cavity is affected by the nature of the substituents in the distal position, which lead to various distortions of the cone structure. The analysis of the interaction between the calix[4]arene bis-crown derivative (Dc3) and acetonitrile by NMR methods confirms the conformation of the Dc3 cavity.

Molecular modeling studies suggest that Dc3 in the cone conformation provides an optimal scaffold to accommodate a CH_3CN molecule. In the MM2 minimized structure, the Dc3 compound shows a hydrophobic cavity with a size appropriate to receive a guest molecule. The following atomic distances characterize the Dc3 cavity: the calixarene cavity has a distance between quaternary C atoms of 7.8−8.0 Å (upper rim) and 5.4−5.6 Å for the crown ether cavity (lower rim).

NMR data obtained for the Dc3−CH_3CN, the free Dc3, and the Dc3−CH_3CN mixture support the host−guest formation. The CH_3CN molecule is included in the Dc3 cavity and the CN group is directed outside this scaffold.

The values of the generalized order parameters (S^2), determined by carbon relaxation rates, also showed that acetonitrile behaves as a free rotor inside the Dc3 cavity with a reorienta-

tional motion that is almost two orders of magnitude faster than that of the aromatic carbons of Dc3.

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Supporting Information Available: Spectral analysis of Dc3 in $CDCl_3$ solution (dqf-COSY spectrum) and Dc3-acetonitrile host−guest complex in $CDCl_3$ (hetero COSY (^1H, ^{13}C) spectrum). (PDF.) This material is available free of charge via the Internet at http://pubs.acs.org.

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