for 2 h and then poured into ice. The red solid that was obtained was dissolved in acetone and filtered, the acetone was evaporated, and the residue was dried and recrystallized from $CHCl_3$ to give 1.34 g (84%) of colorless crystals: mp above 410 °C; IR (KBr) 1770 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 6.75 (s, 2, ArH), 3.6 (s, 2, $ArCH_2Ar$), 1.25 (s, 9, $C(CH_3)_3$. Anal. Calcd for $C_{78}H_{78}O_{12}F_{18}$: C, 60.45; H, 5.08. Found: C, 59.92; H, 5.34.

5,11,17,23,29,35-Hexakis(1,1,3,3-tetramethylbutyl)-37,38,39,40,41,42-hexakis[(trimethylsilyl)oxy]calix[6]arene (8). A slurry of 1.0 g of *p-tert*-octylcalix[8]arene, 6 mL of *N,O*-bis[(trimethylsilyl)-oxy]acetamide, and 50 mL of acetonitrile was refluxed 4 h. The precipitate was collected and recrsytallized from $CHCl_3$ to give 0.81 g (60%) of a white powder: mp 324–327 °C; ^1H NMR (toluene-d_8) δ 6.66 (s, 2, ArH), 3.70 (br s, 2, $ArCH_2$), 1.67 (s, 2, $ArC(CH_3)_2CH_2C(CH_3)_3$), 1.29 (s, 6, $ArC(CH_3)_2CH_2C(CH_3)_3$, 0.71 (s, 9, $ArC(CH_3)_2CH_2C(CH_3)_3$), 0.02 (s, 9, $Si(CH_3)_3$). Anal. Calcd for $C_{108}H_{180}O_6Si_6$: C, 73.47; H, 10.20. Found: C, 74.03; H, 9.93.

37,38,39,40,41,42-Hexakis[(trimethylsilyl)oxy]calix[6]arene (1) was prepared in the manner described above to yield 55% of the product as colorless crystals after recrystallization from $CHCl_3$/MeOH: mp 284–286 °C; ^1H NMR (toluene-d_8) δ 7.35 (t, 1, ArH), 6.95 (d, 2, ArH), 3.70 (s, 2, $ArCH_2Ar$), 0.02 (s, 9, $Si(CH_3)_3$). Anal. Calcd for $C_{60}H_{84}O_6Si_6$: C, 65.93; H, 7.69. Found: C, 67.58; H, 8.10.

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Calixarenes. 15. The Formation of Complexes of Calixarenes with Neutral Organic Molecules in Solution

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Abstract: A study of the interaction, in solution, between calixarenes (as putative host molecules) and a variety of potential guest molecules is reported. By employing the aromatic solvent induced shift (ASIS) values of ^1H NMR resonances of the calixarenes in toluene solution (chloroform as the reference solvent), evidence is adduced for the formation of complexes between certain calix[4]arenes and toluene. The data indicate that the extent of complexation depends on the para substituent of the calixarene; e.g., *p-tert*-butyl- and *p-tert*-amylcalix[4]arene appear to form tighter complexes with toluene than *p*-hydro- and *p-tert*-octylcalix[4]arenes. This correlates with X-ray crystallographic observations on these compounds. Aliphatic amines are shown to interact quite strongly with calix[4]arenes in polar solvents (acetone and acetonitrile), forming complexes that are thought to involve proton transfer from calixarene to amine to produce a calixarene anion and an ammonium cation, followed by ion pairing. Chemical shift and relaxation time (T_1) data, along with measurements of the changes in the free energies of activation for conformational inversion of the calixarenes in the presence of various amines, provide evidence that these are endo-calix complexes.

The calixarenes,[1] which are macrocyclic compounds containing cavities of molecular-sized dimensions, are interesting because of their potential as enzyme mimics. The first step in a faithful enzyme mimic process involves the formation of a complex between the mimic and the substrate, followed then by chemical alteration of the substrate. The present study is concerned with the extent to which calixarenes interact with other molecules to form complexes in solution.

Many of the calixarenes form complexes in the solid state, this property having been observed even before the basic structures of the calixarenes were established. For example, *p-tert*-butylcalix[4]arene (**2**) forms complexes with chloroform,[2] benzene,[3] toluene,[4] xylene,[3] and anisole;[3] *p-tert*-butylcalix[5]arene (**7**) forms complexes with isopropyl alcohol[5] and acetone;[3] *p-tert*-butylcalix[6]arene (**8**) forms a complex containing chloroform and methanol;[2] *p-tert*-butylcalix[7]arene forms a complex containing methanol;[6] and *p-tert*-butylcalix[8]arene (**9**) forms a complex with chloroform.[2] The tenacity with which the guest (substrate) compound[7] is held by the calixarene varies widely among the calixarenes. Whereas the cyclic octamer loses the guest molecule chloroform upon standing a few minutes at room temperature and atmospheric pressure, the cyclic tetramer and cyclic hexamer retain solvent even after many hours of drying under high vacuum at high temperature. The endo calix character of the complexes formed by the cyclic tetramers has been established by the X-ray crystallographic studies of Andreetti et al.[4] which show, for example, that the *p-tert*-butylcalix[4]arene–toluene complex contains the toluene well-imbedded in the center of the calix. The pertinent question in comtemplating the utility of calixarenes as enzyme mimics, however, is whether analogous complexes exist *in solution*. To date, there has been little or no information bearing on this question.

A variety of macrocyclic compounds have been shown to form complexes in solution, including the cyclodextrins,[8] crown ethers,[9] cryptands,[9] and cyclophanes.[11] In many instances where strong

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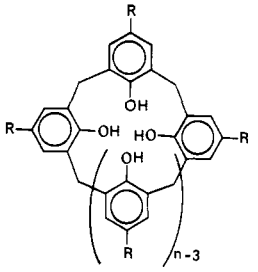


1; n = 4, R = H
2; n = 4, R = $C(CH_3)_3$
3; n = 4, R = $C(CH_3)_2CH_2CH_3$
4; n = 4, R = $C(CH_3)_2CH_2C(CH_3)_3$
5; n = 4, R = $CH_2CH{=}CH_2$
6; n = 4, R = CH_2CH_2OH
7; n = 5, R = $C(CH_3)_3$
8; n = 6, R = $C(CH_3)_3$
9; n = 8, R = $C(CH_3)_3$
10; n = 8, R = $C(CH_3)_2CH_2CH_3$
11; n = 8, R = $C(CH_3)_2CH_2C(CH_3)_3$

complexes are formed the forces responsible (type I) can be attributed to repulsive interactions between the guest molecule and the solvent, e.g. hydrophobic forces in the case of water soluble hosts and lipophobic forces in the case of organic solvent soluble hosts.[12] In other instances, particularly in aprotic solvents, the principal attractive forces (type II) between host and guest arise from Coulombic (charge–charge or dipole–dipole) interactions, hydrogen bond interactions, π interactions, and/or van der Waals interactions. Complexes arising from type I forces have been referred to as "entropically driven" and those arising from type II forces as "enthalpically driven". Generally, complexation is not attributable to a single interaction but to combinations of them. For example, although hydrophobic forces are thought to play a dominant role in cyclodextrin complexation in aqueous solution,[13] van der Waals forces have been cited as the primary source of selectivity in some cases.[14]

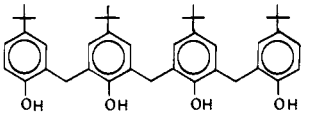
Complexation studies with cyclophanes are especially pertinent to the present work, because the calixarenes are members of the class of $[1_n]$metacyclophanes. A variety of water soluble cyclophanes have been shown to form complexes[15] that in some instances are even stronger than the corresponding cyclodextrin complexes,[16] demonstrating the efficacy of aromatic rings as hydrophobic "walls". Experiments by Koga and co-workers[17] provide the basis for the conclusions that the strongest complexes occur when (1) there is a good fit between host and guest, (2) the hydrophobic area of the host is maximized, and (3) the host preferentially exists in a cavity-containing conformation, a condition also emphasized by Cram in the concept of the "enforced cavity".[18] Organic solvent soluble cyclophanes containing, for example, phenolic rings attached to crown ether moieties,[19] along with the spherands,[20] have been shown to form complexes with alkali cations and amine cations, the attractive forces in these cases being Coulombic and hydrogen bond interactions. The formation of complexes between cyclophanes and neutral guest molecules in nonaqueous solution, however, has been much less extensively investigated. One of the few examples is Whitlock and Jarvi's[21] study of the [8.8]2,6-naphthalenophanes in which they showed that although a water soluble member of this series forms strong complexes in aqueous solution, an organic solvent soluble analogue only forms very weak complexes. The complex with benzene, for example, was determined to have a $K_{association}$ of ca. 2 M^{-1}. A recent study by Reinhoudt and co-workers[22] with crown ethers and neutral, hydrogen bond forming guest molecules has shown that these hosts also have the ability to form complexes in organic solvents but that the association constants are similarly small. It is with this facet of complex formation that the present study is concerned, using calixarenes as the host molecules.

Table I. Aromatic Solvent Induced Shift (ASIS) Values (in ppm) [Chloroform vs. Toluene] for the Linear Tetramer (**12**), Cyclic Tetramer (**2**), Cyclic Hexamer (**8**), and Cyclic Octamer (**9**)[a]

compound	*tert*-butyl	methylene	aryl	hydroxyl
linear tetramer (**12**)	0.065	−0.05	0.30	−0.13
cyclic tetramer (**2**)	0.13	0.01, 0.2	0.08	0.15
cyclic hexamer (**8**)	0.03	0.09	−0.01	0.00
cyclic octamer (**9**)	−0.02	0.02, 0.31	0.05	−0.21

[a] A positive value denotes an upfield shift; a negative value denotes a downfield shift.

Interactions of Calixarenes with Organic Solvents. To probe the interaction of organic solvents with the calixarenes the technique of the aromatic solvent induced shift (ASIS)[23] was employed, using chloroform as the reference solvent, toluene as the aromatic solvent, and tetramethylsilane (Me_4Si) as the internal standard for determining the chemical shift values. The choice of an appropriate reference compound for ascertaining whether the ASIS values indicate complex formation posed some difficulty. The monomeric unit of the *p-tert*-butylcalixarenes, viz. *p-tert*-butylphenol, is not ideal, because its chemical shift values are inherently different from those of its cyclic oligomers. A linear oligomer, although a better reference compound than the monomer, suffers from the possibility of existing in an inter- and/or intramolecularly associated form in solution (which we have referred to as "hemicalixarenes" and "pseudocalixarenes").[24] But, for lack of a better compound we chose the linear tetramer (**12**) as the reference substance, using the chemical shift values of the hydrogens attached to its nonterminal aryl rings. Table I records the differences in chemical shifts in chloroform and toluene solution (ASIS values) for the resonances arising from the *tert*-butyl hydrogens, methylene hydrogens, aryl group hydrogens, and hydroxyl hydrogens of the reference compound **12** as well as the cyclic tetramer, hexamer, and octamer.



12

Association between a noncyclic arene (e.g., the linear tetramer) and the solvent (toluene) places the aromatic rings of solute and solvent face to face. Thus, an upfield shift in the proton resonances of the aryl and *tert*-butyl protons of the solute would be expected. This, in fact, is what is observed, the ASIS values for the linear tetramer being 0.065 and 0.30 for ArH and *tert*-butyl, respectively.

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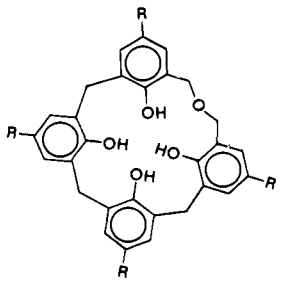
Anything that interferes with the face-to-face interaction should reduce the magnitude of the upfield shift, and anything that reinforces the face-to-face interaction should enhance it. To interpret the ASIS values for the cyclic tetramer, it is postulated that an endo-calix complex is formed in which the toluene is oriented with its methyl group pointed toward the hydroxyl functions at the "bottom" of the cavity. This places a methyl group rather than an aromatic group proximate to the inner faces of the aryl groups of the calixarene, thereby reducing the net diamagnetic anisotropic effect of the solvent (which can now associate only with the aromatic rings of the calixarene at the *outside* faces). On the other hand, the *tert*-butyl groups of the cyclic tetramer–toluene complex *are* proximate to the aryl ring of the guest molecule, which is held more or less rigidly in place. As a consequence, the *tert*-butyl groups experience an even greater diamagnetic anisotropic upfield shift than do those of the reference molecule. The ASIS value for the *tert*-butyl groups of the cyclic tetramer is considerably larger than those of the other cyclic oligomers, suggesting that the cyclic tetramer interacts with the solvent in a fashion different from that of the other cyclic oligomers. This value stands in particularly sharp contrast with the value for the cyclic octamer, which shows a small *downfield* shift and indicates that toluene is even less effectively oriented around the *tert*-butyl groups of this molecule than it is in the reference compound or the cyclic tetramer. This accords with the differences that are observed in the formation of solid-state complexes with these cyclic oligomers, the cyclic tetramer producing a very tight complex with toluene and the cyclic octamer an extremely loose one. That the solid-state complex of the cyclic tetramer is an endo-calix complex has been established by X-ray crystallography;[6] X-ray crystallography of the cyclic octamer,[25] however, shows this molecule to be essentially flat and without a solvent molecule in its center.



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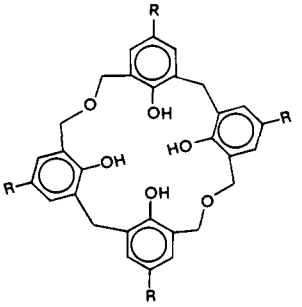

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The ASIS values of the *tert*-butyl groups of the cyclic tetramer in mixtures of chloroform and toluene, as shown in Figure 1, display an approximately linear dependence on the mole fraction of toluene. This is consistent with the formation of a 1:1 complex, although it is not proof for its existence;[26] and it provides a means for calculating an association constant by application of eq 1[27] where Δ_o is the difference in chemical shift between the calixarene in the complexed form (i.e., in pure toluene) and the uncomplexed form (i.e., in pure chloroform), Δ is the difference between the observed chemical shift at a given concentration of guest molecule (i.e., toluene) and that for the calixarene in the uncomplexed state, and $[G]$ is the concentration of the guest molecule. Rearranging eq 1 to eq 2 and plotting $\Delta/[G]$ vs. $\Delta_o - \Delta$ produces a straight line, the slope of which gives a value of K_a of 1.1

$$\frac{1}{\Delta} = \frac{1}{K_a \Delta_o} \cdot \frac{1}{[G]} + \frac{1}{\Delta_o} \quad (1)$$

$$\frac{\Delta}{[G]} = K_a(\Delta_o - \Delta) \quad (2)$$

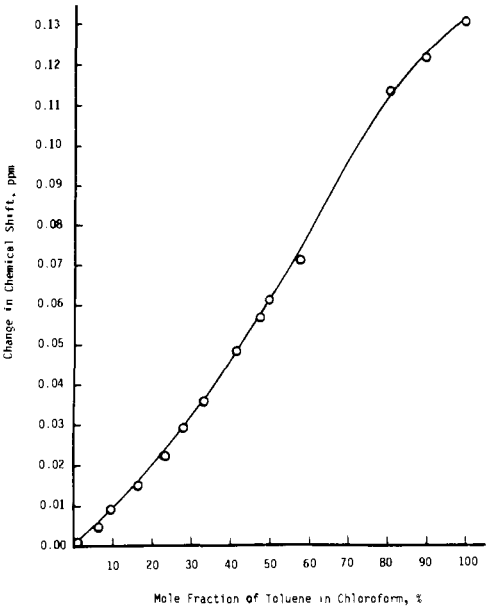
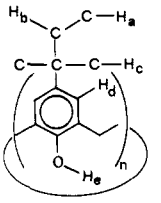
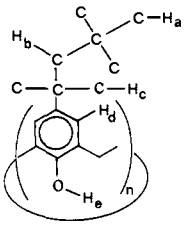


Figure 1. The ASIS values of the *tert*-butyl groups of *p-tert*-butylcalix[4]arene and *p-tert*-butylphenol as a function of the composition of chloroform–toluene solvent mixture.

Table II. Aromatic Solvent Induced Shift (ASIS) Values (ppm) [Chloroform vs. Toluene] for *p-tert*-Amylcalix[4]arene (**3**) and *p-tert*-Octylcalix[4]arene (**4**).

n	H_a	H_b	H_c	H_d	H_e
					
4	0.009	0.073	0.087	0.043	−0.054
8	−0.071	−0.046	−0.041		−0.261
					
4	−0.082	−0.041	−0.022	−0.016	−0.067
8	−0.082	−0.066	−0.050		0.029

[a] A positive value denotes an upfield shift; a negative value denotes a downfield shift.

The ASIS values of *p-tert*-butylcalix[4]arene show a small but linear dependence on temperature between 20 and 100 °C, with a slope of 2.8×10^{-4} ppm/°C. This also is consistent with the formation of a 1:1 complex with toluene, but it is not proof. To seek additional information, the ASIS values for *p-tert*-amylcalix[4]arene (**3**) and *p-tert*-octylcalix[4]arene (**4**)[28] were measured in the hope of finding correlations with the X-ray crystallographic information on these compounds. The results, shown in Table II, reveal that only in the case of *p-tert*-amylcalix[4]arene (**3**) do the ASIS values correspond to upfield shifts, the pattern quite closely resembling that of *p-tert*-butylcalix[4]arene (**2**). The low ASIS value for proton H_a can be rationalized by assuming that the *tert*-amyl group is conformationally oriented so that the terminal methyl group projects away from the "top" of the calix

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(26) Fetizon, M.; Gore, J.; Laszlo, P.; Waegell, B. *J. Org. Chem.* **1966**, *31*, 4047.
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(28) The eight-carbon alkyl group, 2,2,4,4-tetramethylbutyl, will be designated as "*tert*-octyl" in this discussion.

and, therefore, is less susceptible to the diamagnetic anisotropic influence of the complexed toluene. In contrast, all of the ASIS values for *p-tert*-octylcalix[4]arene (**4**) protons correspond to downfield shifts, a pattern suggesting that a complex does not form. The X-ray crystallographic structure of **4** supports this conclusion, for it shows that some of the *p-tert*-octyl groups are folded back into the cavity of this cyclic tetramer to produce a type of self-complexation.[3] *p-tertAmylcalix*[8]arene (**10**) and *p-tert*-octylcalix[8]arene (**11**), like *p-tert*-butylcalix[8]arene (**9**), show only downfield shifts in ASIS values, commensurate with a failure of any of the cyclic octamers to form an endo-calix complex with toluene. *p-tert*-Octylcalix[4]arene (**4**) and *p-tert*-octylcalix[8]-arene (**11**) show surprisingly similar ASIS values, providing further indication that **4** does not form an endo-calix complex with the solvent. The calix[8]arenes might, in fact, serve as better ASIS reference compounds than the linear tetramer. The use of the cyclic octamer as the reference compound would give even larger ASIS values than those shown in Tables I and II for *p-tert*-butylcalix[4]arene and *p-tert*-amylcalix[4]arene.

The conformational properties of the calixarenes have been studied in considerable detail,[29-31] and the free energies of activation for inversion have been measured with a variety of para-substituted calix[4]arenes. The observation[31] that the inversion values for *p-tert*-octylcalix[4]arene are ca. 0.5 kcal/mol lower than those for *p-tert*-butyl- and *p-tert*-amylcalix[4]arene was difficult to explain. That it is not simply a consequence of relative sizes of groups (e.g., a ponderal effect) was demonstrated by the fact that the unsubstituted compound calix[4]arene (**1**) shows a similarly low free energy of activation for conformational inversion. In the light of the present interpretation of the ASIS values for these calixarenes, we postulate that the larger free energy of activation for conformational inversion for *p-tert*-butyl- and *p-tert*-amylcalix[4]arenes arises from endo-calix complex formation. *p-tert*-Octylcalix[4]arene and calix[4]arene, on the other hand, do not form similar complexes. The X-ray crystallographic structure of **4**, which shows that it does not form an endo-calix complex in the solid state, has already been cited.[3] The X-ray crystallographic structure of **1**[32] shows that it, too, does not form an endo-calix complex in the solid state. Thus, the ASIS values reinforce the conclusions reached by Andreetti and co-workers on the basis of X-ray studies of the crystalline calixarenes that (a) the unsubstituted calix[4]arene (**1**) fails to form an endo-calix complex, possibly because its cavity is too shallow, (b) the *p-tert*-octyl-substituted calix[4]arene (**4**) fails to form an endo-calix complex because the para substituent folds back into the calix, and (c) the *p-tert*-butyl-substituted calix[4]arene (**2**) forms a tight endo-calix complex, the *tert*-butyl groups extending the depth of the cavity while not being able to fold back into it.

Selective Complexation of Guest Molecules. *p-tert*-Butylcalix[4]arene (**2**) appears to show little or no selectivity in complexing chloroform and toluene, as indicated by the data in Figure 1 and by the fact that strong endo-calix complexes are formed with both of these solvent molecules in the solid state. With one exception, as discussed in the next section, attempts to find guest molecules that can form endo-calix complexes with a calix[4]arene in chloroform solution have been unsuccessful. The problem apparently arises from the fact that the putative guest compounds do not form complexes that are strong enough to "out-compete" the chloroform to an extent sufficient to make the guest detectable by spectral means. The compounds tested included anisole, nitrobenzene, *p*-xylene, bromobenzene, (trichloromethyl)benzene, (trifluoromethyl)benzene, acetone, *tert*-butylcyclohexanol, *p-tert*-butylphenol, benzonitrile, acetonitrile, (trimethylaceto)nitrile, and phenylacetylene. The host molecules used were *p*-allylcalix[4]arene (**5**) and its tetra-*p*-toluenesulfonate, the latter being chosen because it is known to be fixed in the cone conformation and, therefore, to possess an "enforced cavity".[33] Concentrations up to 10% of the "guest" molecules were used, and changes in ^{1}H NMR spectral shifts and coalescence temperatures for conformational inversion in the case of the parent calixarene were sought. In no case, however, were significant changes observed.

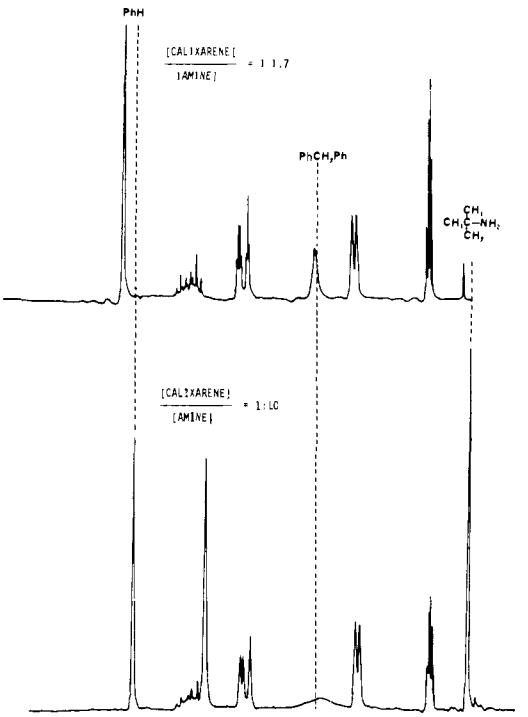

Figure 2. ^{1}H NMR spectra of mixtures of *tert*-butylamine and *p*-allylcalix[4]arene in acetonitrile-d_3 solution at 30 °C.

p-2-Hydroxyethylcalix[4]arene (**6**) and its *p*-toluenesulfonate are soluble in a 3:1 mixture of acetonitrile and water. It was hoped that complex formation might be demonstrated in these cases as the result of the lipophobic forces that presumably account for the formation of complexes of cyclodextrins in Me_2SO solution. However, the ^{1}H NMR spectra of CH_3CN/H_2O solutions of **6** (or its tosylate) in (trichloromethyl)benzene, toluene, chloroform, benzonitrile, or *p*-nitrophenol showed no significant changes. Similar results were obtained when 3:1 Me_2SO/H_2O was used as the solvent. The failure to observe complex formation may again arise from competition between the solvent components (H_2O, CH_3CN, Me_2SO) and the putative guest molecules for complexation with the calixarenes.

Complexes of Calixarenes and Amines. In addition to the compounds discussed in the previous section, *tert*-butylamine was also tested as a potential guest molecule. In this case an observable change in the ^{1}H NMR spectrum was discerned, a result that might have been anticipated from the literature reports on the interactions between phenols and aliphatic amines.[34] Upfield shifts in the resonance positions of the aryl and hydroxyl protons of phenols dissolved in Me_2SO solution containing an amine have been interpreted in terms of phenol–amine complexes.[35]

p-Allylcalix[4]arene (**5**) was chosen as the host molecule for the studies of amine complexation, because it is more soluble in organic solvents than most of the other calixarenes. The ^{1}H NMR spectra of mixtures of **5** and *tert*-butylamine are significantly

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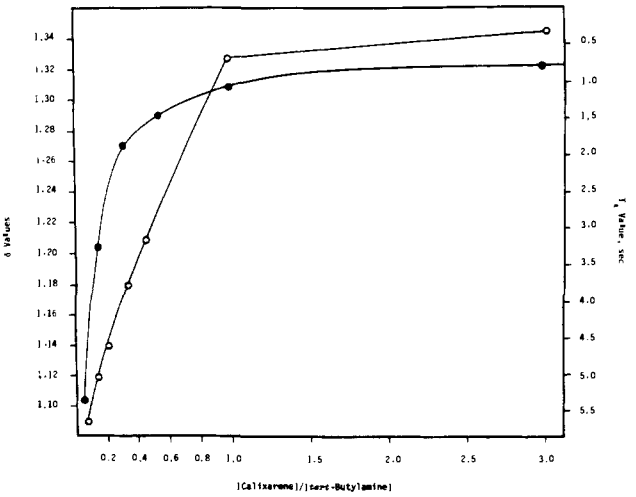

Figure 3. Chemical shifts and relaxation times of the *tert*-butyl protons of *tert*-butylamine as a function of the calixarene/amine ratio (δ values plotted as ●; T_1 values plotted as ○).

different from the spectra of the pure compounds, and the chemical shifts are dependent on the composition of the mixture, as shown in Figure 2 for two different ratios of amine and calixarene. The resonance arising from the *tert*-butyl protons of the amine changes from δ 1.06 in the absence of calixarene to δ 1.37 in the presence of an excess of calixarene; the resonance arising from the amine protons is shifted downfield in the presence of the calixarene and becomes appreciably broadened, suggesting that exchange between the amine protons and the hydroxyl protons of the calixarene occurs at a rate that is within the NMR time scale. From the peak separation and the width of the absorption band it is estimated that the rate of exchange is approximately 10^4 s^{-1}.

Reciprocally, the ^1H NMR pattern of the calixarene also changes in the presence of *tert*-butylamine. The aryl resonances are shifted upfield from δ 7.1 to 6.7, the methylene resonances change from a broad singlet to an unresolved doublet, and the hydroxyl resonance is shifted upfield and is broadened; the resonances arising from the allyl group are shifted only slightly.[36] These spectral changes leave no doubt that the calixarene and *tert*-butylamine are interacting, and the behavior of the hydroxyl and amine group resonances suggests that proton transfer is occurring. This conclusion is reinforced by the observation that a mixture of *tert*-butylamine and picric acid shows a downfield shift of the *tert*-butyl protons to δ 1.34, which is almost the same as that for the calixarene–amine solution. Similarly, a mixture of the calixarene and an excess of NaOH shows a ^1H NMR pattern very similar to that of the calixarene in the calixarene–amine solution. Further confirmation that proton transfer is an important feature of the process is the fact that the *p*-toluenesulfonate of *p*-allylcalix[4]arene affects the resonances of the *tert*-butylamine protons to a much lesser extent than the parent calixarene **5**. A plot of the change in the chemical shift of the *tert*-butyl resonances of *tert*-butylamine as a function of the calixarene/amine ratio, as illustrated in Figure 3, shows that the magnitude of the shift increases until a 1:1 mol ratio of calixarene and amine is reached, at which point the addition of more calixarene has little effect. Similarly, changes in the spectrum of the calixarene reach a maximum at a 1:1 mol ratio, and no further changes occur as more amine is added.

p-Allylcalix[4]arene is not unique in its interaction with *tert*-butylamine; other calix[4]arenes behave in an analogous fashion, shifting the methyl resonances of *tert*-butylamine downfield about δ 0.28 in those cases where the solubility of the calixarene permits

(36) The pattern of the resonances arising from the $=CH_2$ protons of the allyl groups shows a strong temperature dependence both in the absence and presence of amine. At low temperature it appears as a pair of doublets of triplets; at room temperature it appears as three unresolved envelopes; at high temperature it again appears as a pair of doublets of triplets. This suggests that there is restricted conformational motion of the allyl groups at low temperatures.

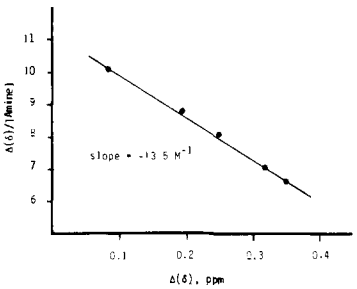

Figure 4. Relaxation times (T_1) of the methyl resonances of *tert*-butylamine measured at various calixarene/amine ratios.

Table III. Relaxation Times (T_1) of the Methyl Protons of *tert*-Butylamine in the Presence of Calixarenes, Oxacalixarenes, and Phenols in Acetonitrile Solution

compound	[amine]/[compd]	T_1, s
none	pure amine	5.25
p-allylcalix[4]arene	50:1	3.5
	5:1	2.41
	1:1.2	0.79
p-toluenesulfonate of *p*-allylcalix[4]arene	1:2	2.45
2,6-dimethyl-4-allylphenol	1:2	4.85
p-nitrophenol	1:11	1.33
2,4,6-trinitrophenol	1:11	1.21
p-*tert*-butyldihomooxacalix[4]arene	1:2	1.5
p-*tert*-butyltetrahomodioxacalix[4]arene	1:2	1.4

it to be used in sufficiently high concentration. Reciprocally, the resonances of the calixarene protons are shifted in the presence of an excess of *tert*-butylamine. For example, the aryl protons of *p*-*tert*-butylcalix[4]arene are shifted upfield by ca. δ 0.2, while the *tert*-butyl protons are shifted downfield by ca. δ 0.01. These data suggest that all of the calix[4]arenes interact with *tert*-butylamine in a similar fashion. Nor is the phenomenon limited to the cyclic tetramers, for the linear tetramer **12**, the calix[6]arenes, the calix[8]arenes, and simple phenols such as 2,6-dimethyl-4-allylphenol (chosen as a monomeric analogue of the *p*-allylcalixarenes) and *p*-nitrophenol (chosen because of its acidity) also have an effect. In these cases, however, 1:1 ratios lead to smaller shifts, and considerably higher ratios of compound to *tert*-butylamine are necessary for the same magnitude of shifts as those of the calix[4]arenes to be observed. *p*-Nitrophenol, for example, requires an 11-fold excess of the phenol to cause a shift of δ 0.28 in the resonances of the methyl groups of *tert*-butylamine, and 2,6-dimethyl-4-allylphenol requires a 34-fold excess.[37]

In contrast to the calixarenes, the oxacalixarenes shift the methyl resonances of *tert*-butylamine downfield only δ 0.17 in the case of *p*-tert-butyldihomooxacalix[4]arene (**13**) and δ 0.14 in the case of *p*-*tert*-butyltetrahomodioxacalix[4]arene (**14**). The resonances of the amine and hydroxyl hydrogens appear as a single broad peak, again indicating that proton exchange is occurring. The aryl resonances of the oxacalixarenes are shifted upfield, the methylene resonances are broadened, and the *tert*-butyl resonances show a very small upfield shift. While the chemical shifts observed with **13** and **14** in the presence of *tert*-butylamine are consistent with the idea that proton transfer takes place to form the *tert*-

(37) The acidities of two calix[4]arenes have been measured in aqueous solution by Böhmer and coworkers.[38] The pK_1 for 5,11,17-trimethyl-23-nitro-25,26,27,28-tetrahydroxycalix[4]arene is 6.0; the pK_1 for 5,17-dimethyl-11-*tert*-butyl-23-nitro-25,26,27,28-tetrahydroxycalix[4]arene is 4.3. These values are lower than that for *p*-nitrophenol itself, presumably the result of stabilization of the monoanion by intramolecular hydrogen bonding. Böhmer's group has shown, however, that the cyclic structure is not essential for this acidity-enhancing effect, for certain linear oligomers behaved in a similar manner.[39] The cyclic tetramer is ideally constituted for effective intramolecular hydrogen bonding and the consequent increase in acidity over its monomeric counterpart. This effect is expected to be manifested in nonaqueous solution as well, perhaps to a considerably greater degree.

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butylammonium cation, the shift of the methyl protons of the amine is much smaller than that of the calixarenes, the linear tetramer, or the monomeric phenols. This can probably be ascribed to incomplete proton transfer in the case of the oxacalixarenes, producing a complex in which hydrogen bond forces play a dominant role; this implies that the oxacalixarenes are weaker acids than the calixarenes. Or, the geometry of the complex might be such that the methyl groups of *tert*-butylamine are proximate to the aryl rings of the oxacalixarenes.

To provide further insight into the nature of the calixarene–amine complex the relaxation times (T_1) of the methyl resonances of *tert*-butylamine were measured at various calixarene/amine ratios. The results, as shown in Figure 4, parallel the chemical shift data quite closely, the greatest decrease in the magnitude of the value occurring when the concentration of the calixarene is equal to or greater than that of the amine. It is instructive to compare the limiting value of 0.79 s for T_1 in this system with the values obtained with various other compounds as shown in Table III. All of these other values are larger, indicating that *p*-allylcalix[4]arene interacts more strongly with *tert*-butylamine than do the other compounds tested. That the decrease in the relaxation time is not due solely to protonation is indicated by the experiment with picric acid in which T_1 is reduced only to 1.21 s. The quantitative differences shown in Table III suggest that there may also be qualitative differences, e.g., that in the case of *p*-allylcalix[4]arene an endo-calix complex forms, binding the *tert*-butylamine more tightly than in the other cases. As noted above, the ^{1}H NMR resonances arising from the methylene groups of a calix[4]arene appear as a singlet at 20 °C in acetonitrile solution in the absence of *tert*-butylamine but as an unresolved doublet in the presence of an excess of amine. This indicates that the rate of conformational inversion is affected by interaction with the amine. A study of the temperature dependence of the ^{1}H NMR spectrum of the calixarene–amine complex, using the techniques described in the accompanying article,[31] provides a coalescence temperature and a free energy of activation for conformational inversion. With use of this as a probe for ascertaining the extent of the calixarene–amine complex formation, the effects of *tert*-butylamine on three calixarenes were tested. It was found that *tert*-butylamine (a) raises the coalescence temperature of *p*-allylcalix[4]arene from 10 to 35 °C (corresponding to an increase in the free energy of activation from 13.5 to 14.7 kcal/mol) and (b) raises the coalescence temperature of calix[4]arene from 2 to 28 °C (corresponding to an increase in free energy of activation of 13.4 to 14.4 kcal/mol).[40] *p-tert*-Butylcalix[4]arene is too insoluble in acetonitrile to allow measurements to be made for the uncomplexed calixarene, but in the presence of *tert*-butylamine it becomes much more soluble and shows a coalescence temperature of 50 °C (corresponding to a free energy of activation of 15.4 kcal/mol).

In addition to *tert*-butylamine a number of other amines have also been tested, using *p*-allylcalix[4]arene as the "host" molecule. The results, shown in Table IV, reveal a significant difference among the amines, ranging from undecylamine (which has a considerable effect on the conformational inversion rate) to aniline (which appears to have virtually no effect). The major factor accounting for these differences is thought to be the basicity of the amine, those amines that show an appreciable effect having basicities on the order of 10^5 times greater than that of aniline. However, other effects, probably steric in nature, also appear to play a part. Neopentylamine, for example, is measurably less effective than *tert*-butylamine in spite of the fact that the basicities of these two amines are very similar. This difference can be rationalized by assuming that the calixarene and amine form an endo-calix complex and that the preferred orientation of the amine in this complex places the three hydrogens of the ammonium nitrogen proximate to the oxygens in the bottom of the cavity,

(40) The chemical shift difference between the methylene doublets is considerably greater in the presence of amine, thus accounting for a 1.2 kcal/mol change in free energy corresponding to a 25 °C change in coalescence temperature.

Table IV. Coalescence Temperatures and Free Energies of Activation for the Conformational Inversion of *p*-Allylcalix[4]arene in the Presence of Amines

amine	[amine]/[calixarene]	solvent	T °C	$\Delta G^{\ddagger}$, kcal/mol
none		$CDCl_3$	38	15.02
none		CD_3CN	10	13.52
none		acetone-d_6	10	13.52
(NaOD)		CD_3CN	42	15.1
tert-butylamine	2:1	acetone-d_6	28	
tert-butylamine	2:1	CD_3CN	32	
tert-butylamine	4:1	acetone-d_6	35	14.67
tert-butylamine	4:1	CD_3CN	36	14.72
tert-butylamine	4:1	$CDCl_3$	30	14.7
tert-butylamine	20:1	acetone-d_6	35	
neopentylamine	4:1	acetone-d_6	15	13.57
neopentylamine	4:1	CD_3CN	19	13.58
neopentylamine	20:1	acetone-d_6	28	
neopentylamine	20:1	CD_3CN	33	
neopentylamine	40:1	acetone-d_6	33	
tert-amylamine	4:1	acetone-d_6	38	
n-butylamine	4:1	acetone-d_6	33	
n-butylamine	4:1	CD_3CN	33	
undecylamine	4:1	acetone-d_6	42	15.1
undecylamine	4:1	CD_3CN	44	
adamantylamine	4:1	acetone-d_6	36	
diethylamine	4:1	CD_3CN	24	
triethylamine	4:1	CD_0CN	23	
quinuclidine	4:1	CD_3CN	29	
aniline	10:1	acetone-d_6	11	

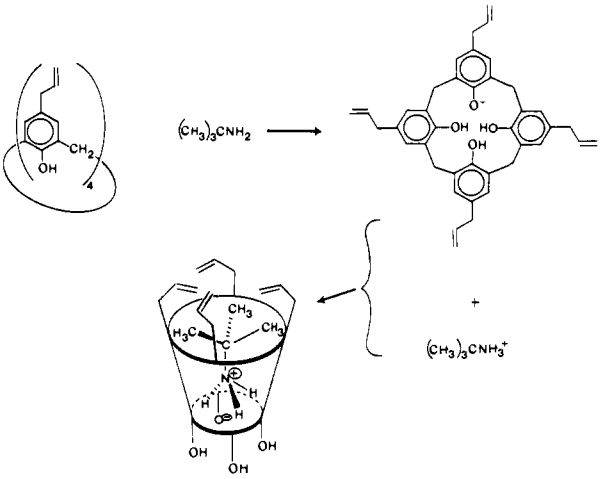

Figure 5. Complex formation between *tert*-butylamine and *p*-allylcalix[4]arene.

producing a "tripod-like" association as shown in Figure 5. In the case of *tert*-butylamine, the *tert*-butyl group sits quite comfortably in the middle of the calix; in the case of neopentylamine, however, the "bend" in the neopentyl group throws the *tert*-butyl portion against the side of the calixarene and makes the "tripod"-like association less effective. Diethylamine and triethylamine also are somewhat less effective than *tert*-butylamine, although not to the extent that might be anticipated if a steric factor of the sort imputed to neopentylamine is operative. The slightly higher coalescence temperature for quinuclidine might be ascribed to a better fit of the symmetrical quinuclidine ring in the cavity of the calixarene; the higher value for undecylamine might arise from the ability of its long hydrocarbon chain to form a coil and fill the cavity.

Solvent properties play an important role in the calixarene–amine interaction. For example, *tert*-butylamine raises the coalescence temperature of *p*-allylcalix[4]arene in both of the polar solvents acetone and acetonitrile but lowers it in the nonpolar solvent chloroform. In contrast to the large downfield shifts of the resonance of the *tert*-butyl protons of the amine that are observed for the calixarene–amine complexes in acetone and acetonitrile, only a small downfield shift (δ 0.01) is observed in

chloroform. Nevertheless, the T_1 value for the *tert*-butyl protons in this system is reduced from 3.2 to 0.8 s, suggesting that a complex is formed but that it is different in structure from the ones that exist in the more polar solvents.

Experimental Section

Determination of Dissociation Constants for Calixarene–Amine Complexes. In a typical determination a 0.0542 M solution of *p*-allylcalix[4]arene in CD_3CN was used to prepare solutions containing measured excesses of *tert*-butylamine. The ^1H NMR spectra were measured on a JEOL FX-100 spectrometer, using 200 scans with a 90° pulse and a 14 s delay. The concentration of the amine in a given solution was determined by a comparison of the integrated intensity of its methyl resonance with that of the aryl resonance of the calixarene.

Determination of Relaxation Times (T_1). Solutions of *p*-allylcalix[4]arene (0.005 M) in CD_3CN containing various concentrations of *tert*-butylamine were degassed at 0.1 mmHg pressure with six freeze–thaw cycles. The T_1 values of the proton resonances were obtained at 100 MHz and 25 °C by using the inversion recovery method.[41] To determine the T_1 of a given resonance a series of 11 spectra (each consisting of 25–100 scans) was obtained by using 180°–τ–90° pulse sequences with a delay of 5 times T_1 between pulse sequences. The time between pulses (τ) was varied between 0.5 and 1.5 times the T_1. The T_1 values were determined from semilogarithmic plots of the intensity of the resonance vs. τ. In all cases the deviation from linearity was less than 0.0005 s; the T_1 values are accurate to ±0.01 s.

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Proposed Structure of Heme *d*, a Prosthetic Group of Bacterial Terminal Oxidases

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Abstract: Heme *d* has been isolated from the purified terminal oxidase complex of *Escherichia coli* strain MR43L/F152. After conversion to the metal-free methyl ester form, termed chlorin *d*, its structure was investigated. It was determined to be a chlorin, that is, a porphyrin with one pyrrole ring saturated so that the β carbons have sp^3 hybridization. Substituents were identified by means of visible, infrared, mass spectrometric, and ^1H NMR spectroscopy. The configuration of substituents was assigned by means of nuclear Overhauser enhancements between nearest neighbors and by the differential effects of a lanthanide shift reagent. The proposed structure of chlorin *d* contains the substituents 1-methyl, 2-vinyl, 3-methyl, 4-vinyl, 5-methyl, 5-hydroxyl, 6-(γ-lactone), 7-propionyl methyl ester, and 8-methyl. Ring C is the saturated pyrrole, and the 6-substitutent is an unusual spiro-γ-lactone.

In 1928 a pigment was observed in cells of *E. coli* and *Shigella dysenteriae* grown aerobically that appeared to be an unusual cytochrome with a pronounced red shift of the α band of the visible spectrum to the 630-nm region.[1] Further work on the respiratory electron transport chain of certain bacteria confirmed the existence of this cytochrome which came to be called cytochrome a_2. It has been detected in diverse bacteria including *Azotobacter*, *Proteus*, *Acetobacter*, *Salmonella*, *Bacillus*, *Pseudomonas*, and *Haemophilus*. By in vivo experiments as well as isolation and characterization of purified enzymes, the function of cytochrome a_2 was established. It functions in the aerobic respiratory metabolism of certain bacteria as a terminal oxidase reducing oxygen to water. Since about 1970, this cytochrome and its prosthetic group heme have been termed cytochrome *d* and heme *d* to avoid confusion with cytochrome aa_3 and the prosthetic group heme *a*, because it became clear that different chemical structures pertained.

In 1956 Barrett[2] proposed a structure for heme *d* as the Fe chelate of 7,8-dihydroprotoporphyrin IX, with some ambiguity as to whether the 2,4-vinyl groups were instead hydroxyethyl groups. This proposal was based upon derivatization chemistry as well as comparisons of chromatographic behavior and visible spectra with models. The main source of material was cells of *Aerobacter Aerogenes*, but chromatographic and visible spectral comparisons indicated that the pigment was identical with that found in *Azotobacter vinelandii*, *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*.

Several considerations have prompted a fuller structural investigation on heme *d*. A deeper understanding of the terminal oxidase function of the host enzyme will require precise structural features for the prosthetic group active site. A recent structure determination on heme d_1 from bacterial dissimilatory nitrite reductases revealed that heme d_1 possesses a novel structure very

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(1) A review of the history of cytochromes *d* with references to the original literature may be found in the following: Lemberg, R.; Barrett, J. "Cytochromes"; Academic Press: New York, 1973; pp 8–14 and 233–240. In this paper the following terminology will be employed. "Heme" is a general term referring to the iron chelate form regardless of the oxidation state of either the central iron or the organic macrocycle. "Chlorin" refers to a porphyrin with one pyrrole that has been saturated so that the β carbons are now quaternary. "Protoheme" refers to Fe protoporphyrin IX without implying any information about the Fe oxidation state or the identity of axial ligands. "Heme *b*" is a general term for a noncovalently associated prosthetic group that characteristically gives an α band in the ferrous state near 560 nm. It is often taken as synonymous with protoheme, but in fact, this requires structural confirmation. "Cytochrome" refers to a heme associated with a polypeptide chain, whether the polypeptide is a stand alone protein or a subunit of a complex enzyme.

(2) Barrett, J. *Biochem. J.* **1956**, *64*, 626–639.

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