pK_a Determination of Water-soluble Calix[4]arenes

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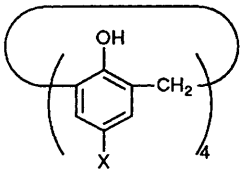
Neutral, water-soluble 5,11,17,23-tetrakis[bis-(2-hydroxyethyl)aminosulphonyl]calix[4]arene-25,26,-27,28-tetraol and 5,11,17,23-tetranitrocalix[4]arene-25,26,27,28-tetraol have been synthesized and the pK_a values of the OH groups determined in an aqueous system.

Calixarenes are cyclic oligomers made up of phenol units. It has been established that the phenolic hydroxy groups appended on the lower rim form strong intramolecular hydrogen bonds, which serve as the main driving force for the stabilization of the 'cone' conformation.[1-5] The presence of such strong intramolecular hydrogen bonds has been demonstrated by ^{1}H NMR and IR spectroscopic methods: for example, the δ_H value for the OH group is *ca.* 10 ppm and the ν_{OH} value shifts to 3100 cm^{-1}.[1,2,6,7] In acyclic analogues, on the other hand, the δ_H value is 7–9 ppm and the ν_{OH} value 3200–3300 cm^{-1}.[7] These findings suggest that the pK_a values for calixarenes would be quite different from those for acyclic analogues. Unfortunately, so far, studies on the pK_a values of calixarenes have been rather limited.[7-10] Böhmer *et al.*[8] synthesized calixarenes containing a *p*-nitrophenol unit and estimated the pK_a by a spectroscopic method. They concluded that the *p*-nitrophenol unit in calix[4]arenes shows nearly the same pK_a as in the acyclic analogues.[8] We previously synthesized water-soluble calix[4]-arenes which have sulphonato groups or 4-trimethylammoniophenylazo groups at the 5,11,17,23-positions and found that the dissociation of the first proton occurs in the very acidic pH region.[9,10] To the best of our knowledge, the phenol moiety in these compounds may be considered as one of the most acidic phenols observed so far. The presence of such a 'superacidic' proton is also supported by X-ray crystallographic studies of tetrasodium 5,11,17,23-tetrasulphonatocalix[4]arene-25,26,-27,28-tetraol.[11,12]

Accurate pK_a determination of calix[4]arenes becomes possible when they are partially water-soluble.[9,10] However, sulphonate and trimethylammoniophenylazo groups, which were introduced to make calix[4]arenes water-soluble, complicated the pK_a measurements: it was quite difficult to isolate the salt-free, water-soluble calix[4]arenes. These observations led us to investigate the pK_a of 'neutral', water-soluble calix[4]arenes, the purification of which can be achieved more easily. Therefore, we have synthesized 5,11,17,23-tetrakis[bis(2-hydroxyethyl)aminosulphonyl]calix[4]arene-25,26,27,28-tetraol (**1a**), 5,11,17,23-tetranitrocalix[4]arene-25,26,27,28-tetraol (**1b**), and their acyclic analogue (**2**$_n$) and determined their pK_a values by photo- and potentio-metric titrations.



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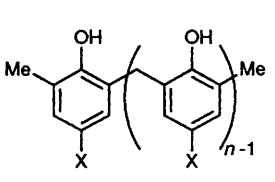

2$_n$

a; X = $SO_2N(CH_2CH_2OH)_2$; **b**: X = NO_2

Experimental

Materials.—The preparation of **1b** has been described previously.[13] Compound **2**$_3$**b** was synthesized according to a literature method.[14]

2,6-*Dimethyl*-4-*bis*(2-*hydroxyethyl*)*aminosulphonylphenol* (**2**$_1$**a**). 2,6-Dimethyl-4-chlorosulphonylphenol (0.50 g, 2.3 mmol) in THF (10 cm^3) was added dropwise to a refluxing solution of diethanolamine (2.0 g, 19 mmol) in THF (20 cm^3) under a nitrogen stream. After 5 h the solution separated into two layers, the upper layer being recovered with a separation funnel. The solution was concentrated *in vacuo* and the residue was dissolved in water (10 cm^3). This was acidified (to *ca.* pH 1) with conc. HCl and the product was salted out from this solution by the addition of NaCl. Yield 27%, m.p. 146–147 °C; ν_{max}(Nujol)/cm^{-1} 3400 (OH) and 1150 and 1320 (SO_2); δ_H(CD_3OD) 2.32 (6 H, s, CH_3), 3.27 and 3.75 (4 H each, t each, CH_2CH_2) and 7.44 (2 H, s, ArH) (Found: C, 54.85; H, 7.35; N, 5.5. $C_{12}H_{19}NO_5$ requires C, 56.02; H, 7.44; N, 5.44%).

2,6-*Bis*[(2-*hydroxy*-3-*methyl*-5-bis(2-*hydroxyethyl*)*amino-sulphonylphenyl*)*methyl*]-4-*bis*(2-*hydroxyethyl*)*amino-sulphonylphenol* (**2**$_3$**a**). 3,5-Bis[(2-hydroxy-3-methylphenyl)-methyl]phenol was sulphonated in a manner similar to that previously described.[13] The sulphonated product was treated with thionyl chloride to yield 2,6-bis[(2-hydroxy-3-methyl-5-chlorosulphonylphenyl)methyl]-4-chlorosulphonylphenol (**3**). The synthetic method has also been described previously.[15] Compound **3** (3.0 g, 4.76 mmol) and diethanolamine were dissolved in DMF (100 cm^3) and the solution was heated at 70 °C with stirring. After 12 h the solution was concentrated *in vacuo*, the oily residue was washed with dil. HCl and water. The product was isolated by column chromatography (Wako gel C-300, isopropyl alcohol). The glassy solid thus obtained was dissolved in methanol and precipitated by the addition of 1 mol dm^{-3} HCl solution. The precipitate (**2**$_3$**a**) was washed with water and dried *in vacuo*. Yield 10%, m.p. 113–117 °C: ν_{max}(KBr)/cm^{-1} 3400 (OH) and 1150 and 1330 (SO_2); δ_H(CD_3OD) 2.29 (6 H, s, CH_3), 3.16 and 3.81 (12 H each, t each, CH_2CH_2), 4.07 (4 H, s, $ArCH_2Ar$) and 7.49–7.30 (6 H, m, ArH) (Found: C, 48.5; H, 6.05; N, 5.0. $C_{34}H_{49}N_3O_{15}S_3$ requires C, 48.85; H, 5.91; N, 5.03%).

5,11,17,23-*Tetrakis*[*bis*(2-*hydroxyethyl*)*aminosulphonyl*]-*calix*[4]*arene*-25,26,27,28-*tetraol* (**1a**). Tetrasodium 5,11,17,23-tetrasulphonatocalix[4]arene-25,26,27,28-tetrol[12] was treated with thionyl chloride to yield 5,11,17,23-tetrakis(chloro-sulphonyl)calix[4]arene-25,26,27,28-tetraol (**4**). Compound **4** (2.0 g, 2.4 mmol) in THF (120 cm^3) was added dropwise to a solution of diethanolamine (10 g, 95 mmol) in THF at room temperature under a nitrogen stream. After 1 h, the reaction mixture was concentrated to dryness *in vacuo*, the residue being washed with 0.1 mol dm^{-3} HCl solution. The solid residue thus obtained was crystallized from methanol: yield 20%. m.p. >300 °C; ν_{max}(KBr)/cm^{-1} 3100–3400 (OH) and 1140 and 1330

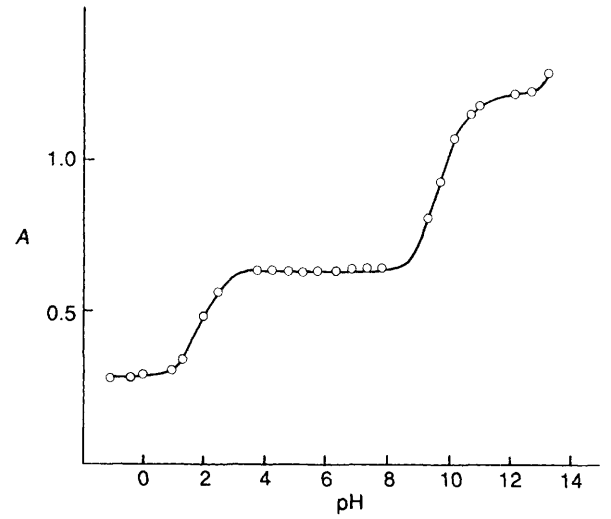

Fig. 1 Photometric titration of **1a** (5.00×10^{-5} mol dm^{-3}): 25 °C, μ = 0.1 with KCl. The wavelength used herein is 280 nm which is the absorption maximum of the phenolate anion.

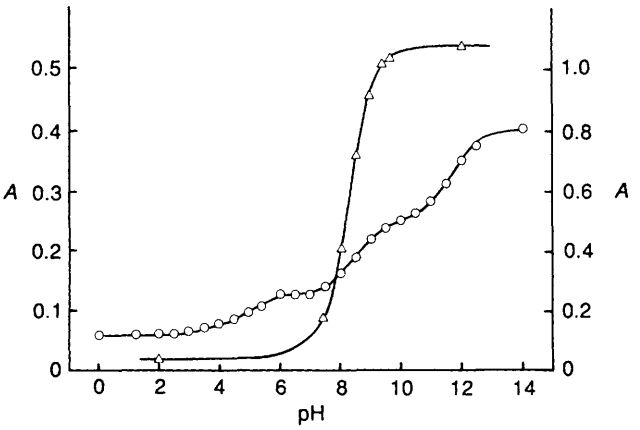

Fig. 2 Photometric titration of $\mathbf{2_1a}$ (△: 6.18×10^{-5} mol dm^{-1}) and $\mathbf{2_3a}$ (○: 1.33×10^{-5} mol dm^{-3}). The titration conditions are recorded in the caption to Fig. 1.

(SO_2); δ_H[$(CD_3)_2SO$; 100 °C] 3.08 (16 H, t, NCH_2), 3.51 (16 H, t, $NCCH_2$), 3.93 (8 H, s, $ArCH_2Ar$), 7.47 (8 H, s, ArH) (Found: C, 45.3; H, 5.45; N, 4.8; S, 10.45. $C_{44}H_{60}N_4O_{20}S_4$ requires C, 48.34; H, 5.53; N, 5.12; S, 11.73%).

Potentiometric pK_a *Determinations of* **1a** *and* $\mathbf{2_n a}$.—The titrations were carried out at 25 °C. The compound was dissolved in 0.01 mol dm^{-3} aqueous HCl and titrated with 0.1 mol dm^{-3} aqueous NaOH as a titrant. The concentration of calix[4]arene was adjusted to 8.0–9.8 $\times 10^{-4}$ mol dm^{-3}. The HCl-free sample was also titrated in the same manner as described above.

Photometric pK_a *Determinations of* **1a** *and* $\mathbf{2_n a}$.—The titrations were carried out in aqueous solution at 25 °C. The concentrations of **1a**, $\mathbf{2_1a}$ and $\mathbf{2_3a}$ were adjusted to 5.00×10^{-5}, 6.18×10^{-5} and 1.33×10^{-5} mol dm^{-3}, respectively. The pH of the solution was adjusted with 0.01 mol dm^{-3} acetate, phosphate, borate and carbonate buffer. The ionic strength of the solution was adjusted to 0.1 with KCl. The absorbance at 280 nm (λ_{max} for phenolate anion) was plotted against pH and the pK_a values determined by analysis of the photometric titration curves.

Potentiometric pK_a *Determinations of* **1b** *and* $\mathbf{2_n b}$.—The titrations were carried out in a nitrogen-flushed 85.4 wt% EtOH–H_2O solution at 25.0 °C by means of a computerized potentiometric titration device described previously.[16] Concentrations of the titrands were kept below 0.001 mol dm^{-3}. Titrants were *ca.* 0.03 mol dm^{-3} solutions of tetrabutylammonium hydroxide in 85.4 wt% EtOH–H_2O. For the calibration of the combined glass/silver–silver chloride electrode (Methrohm, 6.0203.000) buffers as described by Bates were used.[17] The titrations were carried out at least in duplicate.

All calculations were performed using the SUPERQUAD software[18] on a PDP11/84 computer. In the case of calixarene **1b** the input model consisted of three dissociation equilibria. The data of three titrations were combined for the pK_a calculations. The final values of σ and ψ^2 were 1.2 and 44. For *p*-nitrophenol the data of two titrations were combined and the final values of σ and ψ^2 were 1.9 and 11.

Photometric pK_a *Determinations of* **1b** *and* $\mathbf{2_n b}$.—The titrations were carried out in 85.4 wt% EtOH/H_2O solution at 25.0 °C. The concentrations of **1b**, $\mathbf{2_1b}$ and $\mathbf{2_3b}$ were adjusted to 1.87×10^{-5}, 2.50×10^{-4} and 2.45×10^{-5} mol dm^{-3}, respectively. The pH of the solutions was adjusted with HCl and Et_4NOH in 85.4 wt% EtOH–H_2O. The solution pH was corrected according to the method in the literature.[19]

Results

Photometric titration of **1a** was carried out at 25 °C in aqueous 0.1 mol dm^{-3} KCl. At pH < 0 the solution pH was adjusted with H_2SO_4 and corrected by Hammett's acidity function.[19] The result is illustrated in Fig. 1. From this titration curve, three pK_a values can be determined *viz.* 1.80, 9.68 and 12.5 (the pK_a value of 12.5 is not as accurate as the other two values). We have carried out potentiometric titrations under the same conditions to estimate the mole equivalents of OH^- consumed for neutralization of the phenol units. The result established that these values correspond to the first, second and third dissociation of the OH groups in **1a**. It can be seen from Fig. 1 that the first dissociation occurs in very acidic regions (pH 1–3). As recorded in Table 1, the pK_a value for the monomeric analogue $\mathbf{2_1a}$ is 8.25. This indicates that the pK_a value for the first dissociation of **1a** (*i.e.*, pK_{a1}) is shifted to the more acidic pH region by 6.45 pK units. The pK_{a1} for the acyclic trimer $\mathbf{2_3a}$ is 4.71 (Fig. 2). As compared with pK_a 8.25 for $\mathbf{2_1a}$, this value is shifted to the acidic pH region by 3.54 pK units. It is evident that the pK_a shift observed for calix[4]arenes is much greater than that observed for the acyclic analogue.

In Fig. 1 a wide plateau exists between pH 4 and 8. This indicates that further dissociation does not occur in this pH range. The next dissociation begins at *ca.* pH 9 and the pK_a value can be estimated to be 9.68 (Fig. 1). Potentiometric titration indicated that 1.5 mole equivalents of NaOH are consumed at pH 9.68. These results support the view that this pK_a corresponds to the dissociation of the second proton (pK_{a2}).

Compound **1b** and its analogues, which were not so soluble in water, were titrated in aqueous 85.4 wt% ethanol. The absorption of **1b** changed intricately as a function of medium pH (Fig. 3). This suggests the formation of strong intramolecular hydrogen bonds between the undissociated *p*-nitrophenol units and the dissociated *p*-nitrophenolate unit. From potentiometric titration (Fig. 4) we could readily determine pK_{a2} 10.9 and pK_{a3} 12.3. Similar pK_a values (pK_{a2} 11.1 and pK_{a2} 12.3) could be also determined from plots of absorbance (λ 345 nm and 430 nm) against pH (data not shown). The pK_{a1}, which is expected to appear at 2–3, could not be determined because **1b** precipitated below pH 3. We thus measured [H^+] of dilute solutions of **1b** to determine the degree of dissociation of **1b**, and theoretically computed the pK_{a1}. The results, together with those for $\mathbf{2_1b}$ and $\mathbf{2_3b}$ are presented in Table 1.

Table 1 pK_a Values of calix[4]arenes and their analogues[a]

Compound	Method[b]	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}
1a	A	1.8 ± 0.3	9.7 ± 0.1	*ca.* 12.5	>14
2_3a	A	4.71 ± 0.05	8.27 ± 0.05	11.62 ± 0.1	
2_1a	A	8.25 ± 0.03			
1b	B	2.9 ± 0.3	10.9 ± 0.1	12.3 ± 0.2	>14
1b	A	n.d.	11.1 ± 0.2	12.3 ± 0.2	
2_3b	B	3.6 ± 0.1	10.6 ± 0.1	*ca.* 12.5	
2_1b	B	8.67 ± 0.03[c]			

[a] The pK_a values for **1a**, **2_3a** and **2_1a** were determined at 25 °C in aqueous 0.1 mol dm^{-3} KCl solution whereas those for **1b**, **2_3b** and **2_1b** were determined at 25 °C in aqueous 85.4 wt% ethanol. [b] Methods A and B denote photititration and potentiometric titration, respectively. [c] Literature value: 9.07; R. Thuaire, *J. Chim. Phys.*, 1972, **69**, 23.

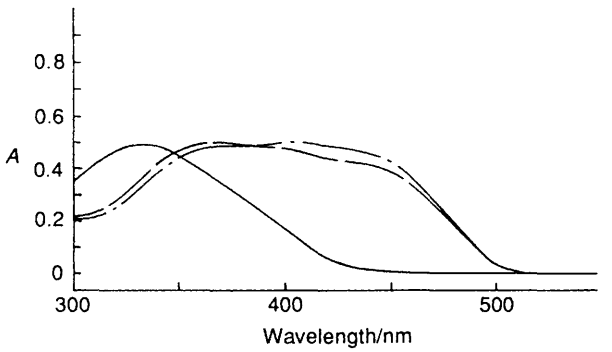

Fig. 3 Absorption spectra of **1b** (2.45×10^{-5} mol dm^{-3}) in 85.4 wt% ethanol solution at 25 °C: — pH 5–9, - - pH 13.0, - · - pH 13.8

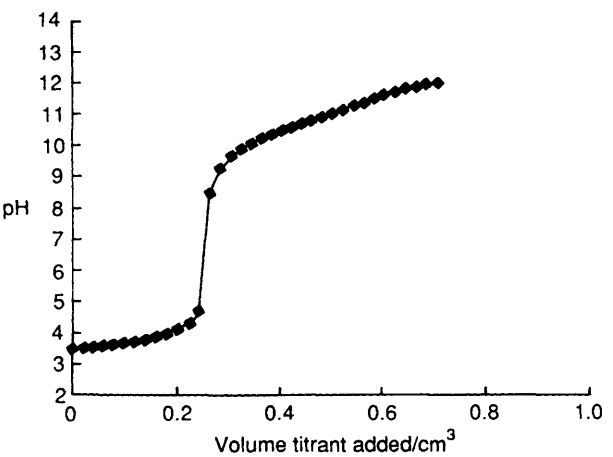

Fig. 4 Potentiometric titration of **1b** in aqueous 85.4 wt% ethanol at 25 °C: 8.87×10^{-6} moles of **1b** (20 cm^3 of solution) were titrated by 0.0289 mol dm^{-3} $Bu_4N^+OH^-$

Discussion

The above described pK_a determinations of **1a** and **1b** reveal that the first deprotonation of calix[4]arenes takes place at very low pH in comparison with the deprotonation of acyclic monophenols **2_1a** and **2_1b**. This is indicative of a relatively acidic compound. The explanation for this 'super-acidic' behaviour of calixarenes seems to be the optimal stabilization of the phenolate anion relative to the undissociated species. Semiempirical (AM1) calculations[5] indicate that the monoanion is optimally solvated by hydrogen bond formation with the two adjacent hydroxy groups, which are in turn stabilized by a bifurcated hydrogen bond with the opposite hydroxy group. This is nicely confirmed by the relatively low pK_a values of the acyclic triphenols **2_3a** and **2_3b**, in which a similar intramolecular hydrogen bonding is possible between the central phenolate anion and the neighbouring phenol moieties. For an optimal comparison the corresponding acyclic tetraphenols should be synthesized and measured. The difference between the first pK_a values of such a compound and a calixarene would reflect the role of preorganization of the hydroxy groups involved in hydrogen bonding. The presence of intramolecular hydrogen bonding in the monoanion was confirmed by photometric measurements (*vide supra*). The AM1 calculations[5] also indicate that favourable charge delocalization can take place in the calixarene monoanion.

The pK_a values corresponding to the second deprotonation are somewhat higher than the pK_a values of the acyclic monophenols **2_1a** and **2_1b**. Computational studies[5] again indicate the possibility of intramolecular hydrogen bonding in the calixarene dianion. However, unfavourable electrostatic repulsion in the calixarene dianion seems to be the dominating effect, resulting in a slightly *decreased* acidity relative to the acyclic monophenols. A similar reasoning holds for pK_{a3} which is even more enhanced. The fourth deprotonation step could not be observed in the present study, but pK_a values higher than 14 are expected.

At this point it should be noted that a remarkable structural similarity exists between the calixarene monoanion and the so-called tetrahedral transition state complex postulated for the proteolytic action of serine proteases.[20–23] In both structures an oxyanion is stabilized by hydrogen bonding with a pair of hydrogen bond donors. In the calixarene the two adjacent hydroxy groups have been predicted to interact with the oxyanion. In serine proteases the tetrahedral oxyanion is stabilized by the main-chain amide groups of Gly 193 and Ser 195. The latter stabilizing interaction by the 'oxyanion hole' is thought to make a major contribution to the required stabilization of the transition state. The pK_a shifts relative to the acyclic compounds **2_1a** and **2_1b** observed for the calixarenes **1a** and **1b** amount to 6–6.45 pK_a units, corresponding to a free energy stabilization effect of 8.4–9.0 kcal mol^{-1} at 298 K.* Assuming that the calixarene monoanion mimics the situation in the serine protease tetrahedral intermediate to some extent, these free energy figures may provide an idea of the transition state stabilization by the oxyanion hole.

Conclusions

The present paper establishes that, as expected on the basis of IR and ^1H NMR spectral data, the dissociation of the first proton in calix[4]arene takes place at unusually low pH (pK_{a1} 1.8–2.9). The remarkable pK_{a1} difference between calix[4]arenes and their acyclic analogue is ascribed solely to the formation of strong intramolecular hydrogen bonds. We believe that the present finding will be helpful in understanding the hydrogen-bond-induced pK_a shift which is frequently observed in enzymic systems.

* 1 cal = 4184 J.

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