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.\(^1\)–\(^3\) The presence of such strong intramolecular hydrogen bonds has been demonstrated by \(^1\)H NMR and IR spectroscopic methods: for example, the \(\delta_v\) value for the OH group is ca. 10 ppm and the \(\delta_{OH}\) value shifts to 3100 cm\(^{-1}\).\(^1\)–\(^2\),\(^6\)–\(^7\) In acyclic analogues, on the other hand, the \(\delta_v\) value is 7–9 ppm and the \(\delta_{OH}\) value 3200–3300 cm\(^{-1}\).\(^1\) 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.\(^5\) 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-trimethylammonio groups, which complicated the \(pK_a\) measurements: it was quite difficult to isolate the \(pK_a\) analogues.* We previously synthesized water-soluble calix[4]-arenes which have sulphonato groups or 4-trimethylammonio groups at the 5,11,17,23-positions and found that the \(pK_a\) of ‘neutral’, water-soluble calix[4]arenes shows nearly the same \(pK_a\) as in the acyclic analogues.\(^9\)\(^,\)\(^10\) However, sulphonate and trimethylammonio groups at the 5,11,17,23-positions and determined their \(pK_a\), values of the OH groups determined in an aqueous system.

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**Experimental**

**Materials.**—The preparation of 1b has been described previously.\(^13\) Compound 2b was synthesized according to a literature method.\(^14\)

2,6-Dimethyl-4-bis-(2-hydroxyethyl)aminosulphonylphenol (2a).—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. 142–147 °C; \(\nu_{OH}\) (KBr)/cm\(^{-1}\) 3400 (OH) and 1150 and 1320 (SO\(_2\)); \(\delta_{CD(OD)}\) 2.12 (6 H, s, CH\(_3\)).

Compound 3 (3.0 g, 4.76 mmol) and diethanolamine were treated with thionyl chloride to yield 2,6-bis[(2-hydroxy-3-methyl-5-bis(2,6-dimethyl-4-chlorosulphonylphenyl)methyl]-4-bis(2-hydroxyethyl)aminosulphonylphenol (3). The synthetic method has also been described previously.\(^15\) Compound 3 (3.0 g, 4.76 mmol) and diethanolamine were 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 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.\(^15\)

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Photometric pKa Determinations of 1a and 2a.—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 × 10⁻⁴ mol dm⁻³. The HCl-free sample was also titrated in the same manner as described above.

Photometric pKa Determinations of 1a and 2a.—The titrations were carried out in aqueous solution at 25 °C. The concentrations of 1a, 2a, and 2b were adjusted to 5.00 × 10⁻³, 6.18 × 10⁻³, and 1.33 × 10⁻³ 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 with 0.01 mol dm⁻³ KCl. At pH 2.6, NaOH in 85.4 wt% EtOH-H₂O solution at 25.0 °C by means of a computerized potentiometric titration device described previously. 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-H₂O. For the calibration of the combined glass/silver-silver chloride electrode (Methrohm, 6.0203.000) buffers as described by Bates were used. The titrations were carried out at least in duplicate.

All calculations were performed using the SUPERQUAD software 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 pKa calculations. The final values of pKa values were 1.2 and 1.4 for p-nitrophenol the data of two titrations were combined and the final values of pKa were 1.9 and 11.

Photometric pKa Determinations of 1b and 2b.—The titrations were carried out in 85.4 wt% EtOH/H₂O solution at 25.0 °C. The concentrations of 1b, 2b, and 2b were adjusted to 1.87 × 10⁻³, 2.50 × 10⁻⁴, and 2.45 × 10⁻⁵ mol dm⁻³, respectively. The pH of the solutions was adjusted with HCl and Et₂NOH in 85.4 wt% EtOH-H₂O. The solution pH was corrected according to the method in the literature.

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 H₂SO₄ and corrected by Hammett's acidity function. The result is illustrated in Fig. 1. From this titration curve, three pKa values can be determined viz. 1.80, 9.68 and 12.5 (the pKa 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 molar 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 pKa value for the monomeric analogue 2a is 8.25. This indicates that the pKa value for the first dissociation of 1a (i.e., pKa₁) is shifted to the more acidic pH region by 6.45 pK units. The pKₕ for the acyclic trimer 2a is 4.71 (Fig. 2). As compared with pKₕ 8.25 for 2a, this value is shifted to the acidic pH region by 3.54 pK units. It is evident that the pKₕ 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 pKa value can be estimated to be 9.68 (Fig. 1). Potentiometric titration indicated that 1.5 molar equivalents of NaOH are consumed at pH 9.68. These results support the view that this pKₕ corresponds to the dissociation of the second proton (pKₕ₂).

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 intrinsically as a function of medium pH (Fig. 3). This suggests the formation of strong intra-molecular hydrogen bonds between the undisassociated p-nitrophenol units and the dissocitated p-nitrophenolate unit. From potentiometric titration (Fig. 4) we could readily determine pKₕ₁ = 10.9 and pKₕ₂ = 12.3. Similar pKₕ values (pKₕ₁ = 11.1 and pKₕ₂ = 12.3) could be also determined from plots of absorbance at 345 nm and 430 nm against pH (data not shown). The pKₕ₁, 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 pKₕ₁. The results, together with those for 2a and 2b are presented in Table 1.
The central phenolate anion and the neighbouring phenol moieties.

For an optimal comparison the corresponding acyclic tetra-

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Discussion

The above described pKₐ 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 2a and 2b. 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 undisassociated species. Semiempirical (AM1) calculations 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 pKₐ values of the acyclic triphenols 2a and 2b, 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 tetra-

phenols should be synthesized and measured. The difference between the first pKₐ 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 monoaion was confirmed by photometric measurements (vide supra). The AM1 calculations also indicate that favourable charge delocalization can take place in the calixarene monoaion.

The pKₐ values corresponding to the second deprotonation are somewhat higher than the pKₐ values of the acyclic monophenols 2a and 2b. Computational studies 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 pKₐ, which is even more enhanced. The fourth deprotonation step could not be observed in the present study, but pKₐ values higher than 14 are expected.

At this point it should be noted that a remarkable structural similarity exists between the calixarene monoaion and the so-called tetrahedral transition state complex postulated for the proteolytic action of serine proteases. 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 pKₐ shifts relative to the acyclic compounds 2a and 2b observed for the calixarenes 1a and 1b amount to 6–6.45 pKₐ units, corresponding to a free energy stabilization effect of 8.4–9.0 kcal mol⁻¹ at 298 K. Assuming that the calixarene monoaion 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]arenes takes place at unusually low pH (pKₐ, 1.8–2.9). The remarkable pKₐ 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 pKₐ shift which is frequently observed in enzymic systems.

* 1 cal = 4184 J.

Table 1: pKₐ Values of calix[4]arenes and their analogues

<table>
<thead>
<tr>
<th>Compound</th>
<th>Method</th>
<th>pKₐ₁</th>
<th>pKₐ₂</th>
<th>pKₐ₃</th>
<th>pKₐ₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>A</td>
<td>1.8</td>
<td>9.7</td>
<td>ca. 12.5</td>
<td>&gt;14</td>
</tr>
<tr>
<td>2a</td>
<td>A</td>
<td>4.70</td>
<td>8.27</td>
<td>11.62</td>
<td>0.1</td>
</tr>
<tr>
<td>1b</td>
<td>B</td>
<td>8.25</td>
<td>10.9</td>
<td>12.3</td>
<td>0.2</td>
</tr>
<tr>
<td>2b</td>
<td>B</td>
<td>0.6</td>
<td>11.1</td>
<td>12.3</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* The pKₐ values for 1a, 2a and 2a were determined at 25 °C in aqueous 0.1 mol dm⁻³ KCl solution whereas those for 1b, 2b and 2b were determined at 25 °C in aqueous 85.4 wt% ethanol. Methods A and B denote phototitration and potentiometric titration, respectively. Literature value: 9.07: R. Thuaire, J. Chim. Phys., 1972, 69, 23.

Fig. 3: Absorption spectra of 1b (2.45 × 10⁻³ 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 cm³ of solution) were titrated by 0.0289 mol dm⁻³ Bu₄N⁺OH⁻.

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References
9 The \( pK_a \) values for 5,11,17,23-tetrasulphonatocalix[4]arene-25,26,27,28-tetraol have recently been re-estimated in water at 25 °C and \( \mu = 0.1 \) with KNO\(_3\): \( pK_{a1} = 3.26 \), \( pK_{a2} = 12.38 \) and \( pK_{a3} = 13.00 \).

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