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Cationic Water-soluble Calixarenes: New Host Molecules which Catalyse Basic Hydrolysis of a Phosphate Ester

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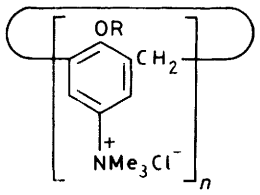
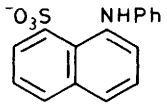
New water-soluble calixarenes (**1**) having cationic charges on the upper rim of the calixarene cavity have been synthesized: they act as efficient catalytic hosts for basic hydrolysis of a phosphate ester which proceeds according to Michaelis–Menten kinetics.

Although calixarenes, cavity-shaped macrocycles, can include small molecules (chloroform, toluene, acetone, *etc.*) in the solid state, few examples of inclusion complexes for calixarenes in solution are known.[1,2] This is in sharp contrast to cyclodextrins which form a variety of host–guest complexes in solution and frequently serve as catalytic hosts and enzyme mimics.[3–5] Thus, the difference between cyclodextrins and calixarenes is ultimately one of solubility: that is, cyclodextrin complexes are formed in water as a result of hydrophobic interactions, whereas calixarenes are practically insoluble.[1] The solubilization of calixarenes in water is, therefore, a prerequisite to any investigation of their complex formation. Earlier we synthesized 'anionic' water-soluble calixarenes and found evidence of complexes in solution.[6,7] Here we describe the first example of 'cationic' water-soluble calixarenes (**1**), which both strongly bind anionic guest molecules and serve as efficient catalysts for ester hydrolysis with Michaelis–Menten kinetics.



(1)



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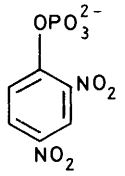
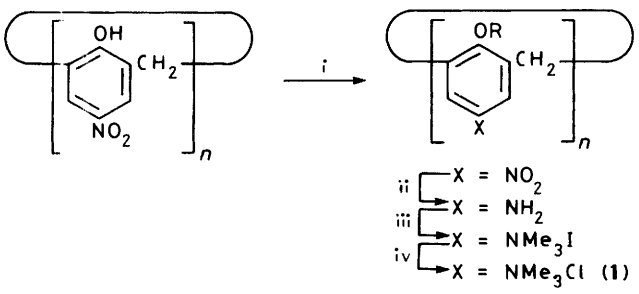


(3)

Calixarenes (**1**) were synthesized from the corresponding *p*-nitrocalixarenes[8] (see Scheme), the products being identified on the basis of i.r. and n.m.r. spectral evidence and elemental analysis.

By the conductivity method[6] the critical micelle concentration (c.m.c.) for (**1**; $n = 6$, R = C_8H_{17}) was estimated to be 1.6×10^{-4} mol dm^{-3} but was not found for (**1**; $n = 4$, R = Me) and (**1**; $n = 6$, R = CH_3) (up to 0.2 mol dm^{-3}). All kinetic and spectroscopic measurements were thus carried out $< 1.6 \times 10^{-4}$ mol dm^{-3} on (**1**; $n = 6$, R = C_8H_{17}).



Scheme. *Reagents:* i, RX; ii, N_2H_4–$FeCl_3$; iii, MeI; iv, ion exchange

Since the fluorescence intensities and emission maxima of anilinonaphthalenes show a strong solvent dependence,[10] we employed sodium 1-anilinonaphthalene-8-sulphonate (**2**) as a fluorescence probe. The fluorescence intensity I at *ca.* 500 nm increased with increasing concentration of (**1**) (excitation 365 nm) (see Figure 1), (**1**; $n = 6$, R = C_8H_{17}) being particularly effective. Benesi–Hildebrand plots (assuming the formation of 1:1 complexes) provided good linear relationships ($r > 0.99$) and we obtained the association constants K (dm^3 mol^{-1}) = 2.6×10^3 for (**1**; $n = 4$, R = Me),† 8.5×10^3 for (**1**; $n = 6$, R = Me), and 3.8×10^4 for (**1**; $n = 6$, R = C_8H_{17}). The emission maximum of (**2**) (520 nm in water and 473 nm in ethanol) correlates linearly with the ethanol concentration in water–ethanol. In aqueous (**1**; $n = 4$, R = Me), (**1**; $n = 6$, R = Me), and (**1**; $n = 6$, R = C_8H_{17}), the emission maximum shifted significantly to shorter wavelengths and was saturated at 510, 480, and 478 nm, respectively. These wavelengths correspond to the solvents containing 25, 88, and 93 vol% of ethanol, respectively. The results indicate that hexameric calixarenes (**1**; $n = 6$, R) have a cavity much more hydrophobic than tetrameric calixarenes (**1**; $n = 4$, R).

Since the foregoing data suggest that (**1**) would act as catalytic hosts for the basic hydrolysis of 'anionic' esters, we tested this idea with 2,4-dinitrophenyl phosphate (**3**), the cationic charges located on the upper rim of the former having the possibility of interacting with the phosphate dianion in the latter. The reaction, carried out at pH 10 and 30 °C and the progress of which was followed by monitoring the appearance of the 2,4-dinitrophenolate absorption band (358 nm), satisfied first-order kinetics for up to three half-lives. As shown in Figure 2, the first-order rate constants (k_{obs}) increased with increasing concentration of (**1**; $n = 6$, R) and were saturated, as clearly seen for (**1**; $n = 6$, R = C_8H_{17}), at *ca.* [(**1**)]/[**3**] = 1.0. Thus, the catalytic rate constants for (**1**; $n = 6$, R = Me) and (**1**; $n = 6$, R = C_8H_{17}) are greater by 21-fold and 46-fold, respectively, than that in bulk water ($k_{obs} = 2.11 \times 10^{-5}$ s^{-1}). The K values

† It is known that *O*-methylation of *p*-t-butylcalix[4]arene results in conformational isomers because steric hindrance of oxygen prevents annulus rotation: D. N. Reinhoudt *et al.*, *J. Am. Chem. Soc.*, 1987, **109**, 4761. The ^{1}H n.m.r. (D_2O) of (**1**; $n = 4$, R = CH_3) gave a pair of doublets at 3.40 and 4.52 p.p.m. for the $ArCH_2Ar$ protons, an indication that it existed in the 'cone' conformation. The 'cone' conformer probably being selectively isolated at some stage in the synthetic route.

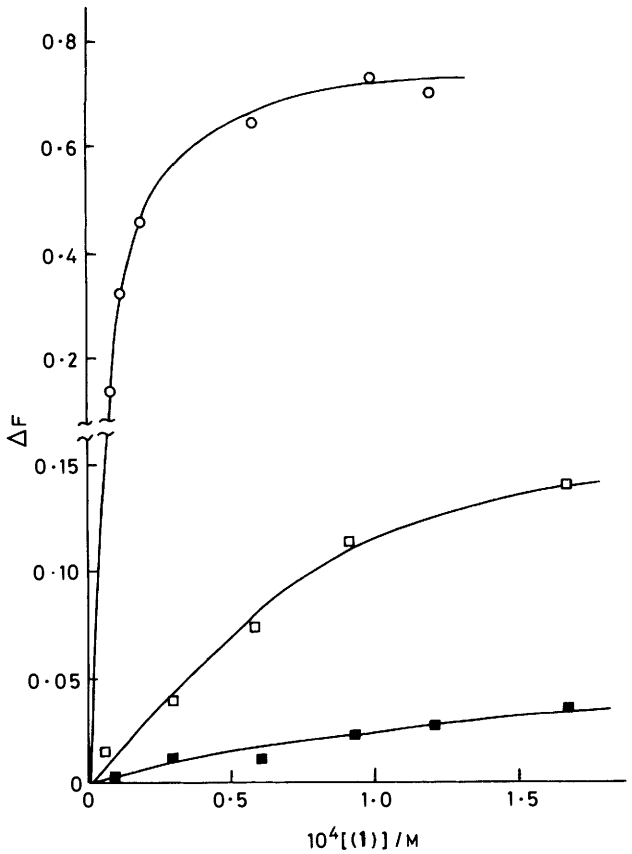


Figure 1. Plots of $\Delta F(=I - I_0)$ *vs.* [(**1**)]: ■ $n = 4$, R = Me; □ $n = 6$, R = Me; ○ $n = 6$, R = C_7H_{18}; [**2**] = 1.00×10^{-5} mol dm^{-3}, excitation 365 nm, 30 °C

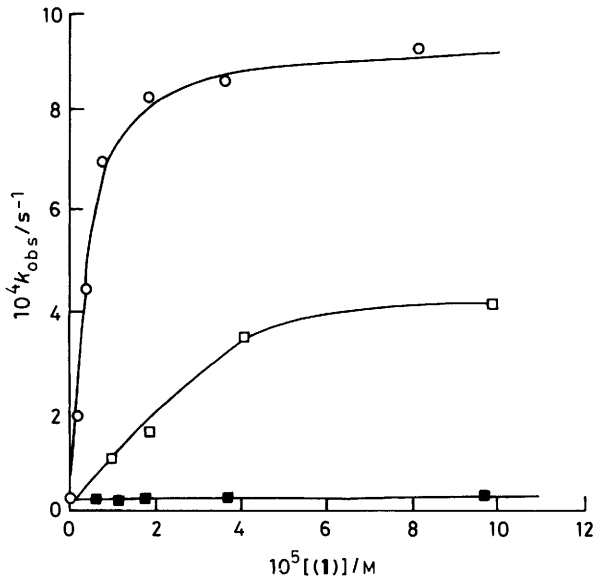


Figure 2. Plots of first-order rate constants (k_{obs}) for the hydrolysis of (**3**) *vs.* [(**1**)]: ■ $n = 4$, R = Me; □ $n = 6$, R = Me; ○ $n = 6$, R = C_8H_{17}; [**3**] = 1.10×10^{-5} mol dm^{-3}, pH 10 with 15 mmol dm^{-3} borate, 0.3 vol% acetonitrile, 30 °C

determined from Figure 2 are 3.56×10^4 dm^3 mol^{-1} for (**1**; $n = 6$, R = Me) and 9.13×10^4 dm^3 mol^{-1} for (**1**; $n = 6$, R = C_8H_{17}). In contrast, (**1**; $n = 4$, R = Me) scarcely catalysed the reaction. This suggests that the molecular architecture of (**1**; $n = 4$, R = Me) is not suitable for the binding of (**3**): possible reasons are (i) the cavity size of (**1**; $n = 4$, R = Me) is too small to accommodate (**3**) and (ii) hexacationic charges placed on the upper rim of (**1**; $n = 6$, R) are complementally pre-organised for multi-point recognition of the tetrahedral $R'PO_3^{2-}$ anion, whereas tetracationic charges placed on the upper rim of (**1**; $n = 4$, R) are mismatched with that anion.

Interestingly, the catalytic activity of (**1**; $n = 6$, R) was efficiently quenched by the addition of phosphoric acid and its derivatives. Since the reaction was scarcely affected by the addition of the same amount of KCl or KBr (~0.01 mol dm^{-3}), the inhibition effect is specific to phosphate derivatives. Thus, the total reaction scheme is expressed by equation (1), where (**I**)

$$\begin{array}{l}(\mathbf{1};\, n = 6, \mathrm{R}) + (\mathbf{3}) \overset{K}{\rightleftharpoons} (\mathbf{1};\, n = 6, \mathrm{R})\cdot(\mathbf{3}) \xrightarrow{k_{cat}} \text{products} \\ \quad (\mathbf{I}) \downarrow \\ [\mathbf{1};\, n = 6, \mathrm{R})\cdot(\mathbf{I}) \end{array} \qquad (1)$$

denotes phosphate derivatives acting as an inhibitor. From plots of k_{obs} *vs.* inhibitor concentration (0.001—0.01 mol dm^{-3}) we estimated K_I for the (**1**; $n = 6$, R = C_8H_{17})-catalysed system to be 220 dm^3 mol^{-1} for phosphoric acid (HPO_4^{2-} at pH 10) and 830 dm^3 mol^{-1} for phenylphosphoric acid ($PhPO_3^{2-}$ at pH 10).

In conclusion, the present study established that (**1**; $n = 6$, R) act as a new class of water-soluble host molecules which exhibit enzyme-mimetic activity for ester hydrolysis. This suggests that the calixarene skeleton serves as a potential candidate for the design of totally synthetic enzyme mimics.*

* A referee of this paper suggested a study of calixarene conformations in water in order to confirm that calixarenes really act as cavity-shaped receptors. At 20 °C in D_2O ^{1}H n.m.r. (400 MHz) spectra of (**1**; $n = 6$, R = Me) and (**1**; $n = 6$, R = C_8H_{17}) gave a singlet peak (4.12 p.p.m.) and multiple peaks (4.06—4.15 p.p.m.), respectively, for the $ArCH_2Ar$ protons. This indicates that (**1**; $n = 6$, R = Me) adopts an 'alternate' (freely-rotating) conformation and (**1**; $n = 6$, R = C_8H_{17}) a more restricted conformation.[1] In the presence of 0.1 mol dm^{-3} K_3PO_4 we observed multiple peaks both for (**1**; $n = 6$, R = Me) (4.02—4.16 p.p.m.) and (**1**; $n = 6$, R = C_8H_{17}) (3.95—4.25 p.p.m.) which were essentially approximated by three pairs of doublets. It is evident, therefore, that PO_4^{3-} strongly interacts with (**1**; $n = 6$, R) to suppress the conformational fluctuation.

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