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NEW WATER-SOLUBLE MACROCYCLES OF THE PARACYCLOPHANE TYPE: AGGREGATION BEHAVIOUR AND HOST-GUEST-INTERACTION WITH HYDROPHOBIC SUBSTRATES

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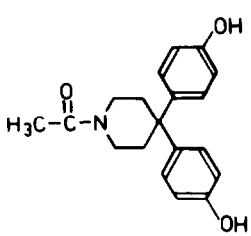
Abstract: The three water-soluble tetraoxa[n.1.n.1.]paracyclophanes 6a-c were synthesized. Their aggregation behaviour was studied by ^{1}H-NMR spectroscopy and light scattering; host-guest-interaction with hydrophobic guests was observed by fluorescence spectroscopy.

Hydrophobic interactions are a major driving force for substrate binding in many biological systems [1]. There is considerable interest in the design of water-soluble macrocycles of the paracyclophane type with hydrophobic cavities of well defined size and shape which allow the selective inclusion of apolar substrates in aqueous solution [2]. In this communication we report on the synthesis and first investigations of the hydrophobic binding ability of a new series of macrocycles in which the quarternary ammonium residues making the molecules water-soluble at room temperature and neutral pH are located remote from the cavity. Thus the cavity represents a hydrophobic binding site not perturbed by strongly hydrated ionic centers, as it would be the case if the quarternary nitrogens are located in the macrocyclic skeleton.



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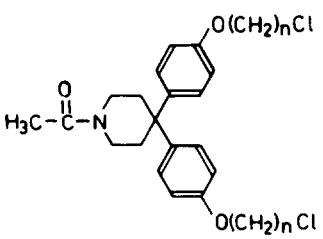
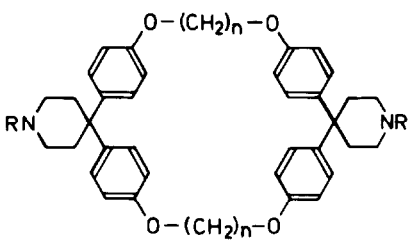
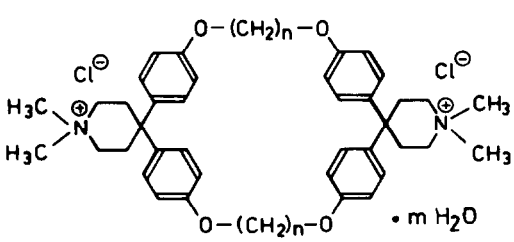


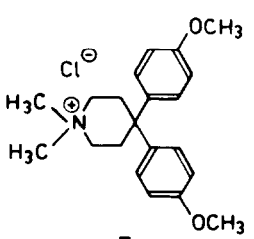
2a-c



3a-c R = $COCH_3$
4a-c = H
5a-c = CH_3



6a n = 2 m = 2.5
6b n = 3 m = 2
6c n = 4 m = 4



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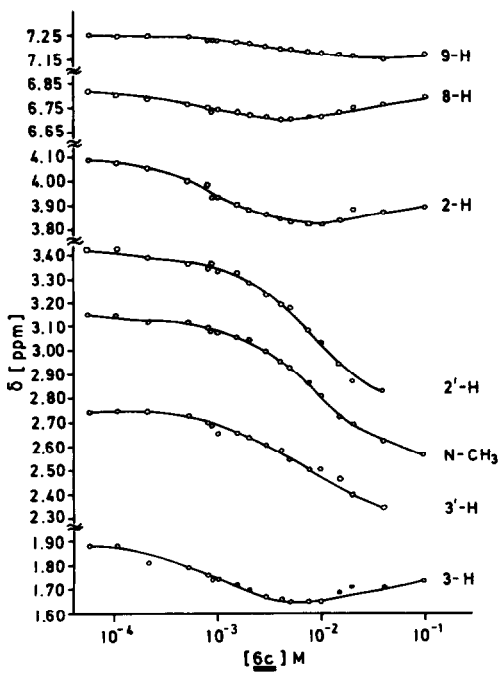
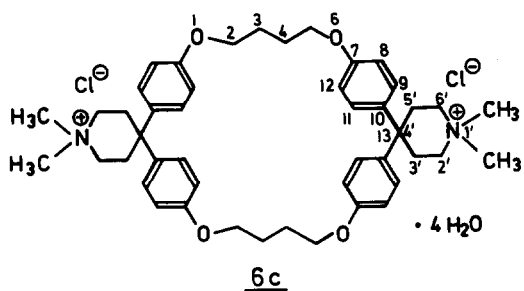
N-Acetyl-4,4-bis(4-hydroxyphenyl)piperidine (1) [3] was obtained from phenol and N-acetyl-4-piperidone in 76 % yield. Cyclization of 1 with 2a - c [prepared from 1 with the corresponding α,ω-dichloroalkanes, 80 - 88 % yield] in n-butanol in the presence of sodium hydroxide provided the tetraoxa[n.1.n.1.]paracyclophanes 3a - c having the desired cavities [13 - 15 % yield]. Hydrolysis of the N-acetyl-residues using sodium hydroxide in 2-methoxyethanol afforded 4a - c [78 - 94 % yield] and Eschweiler-Clarke methylation gave the N-methyl-cycles 5a - c [60 - 90 % yield].

Quarternization was achieved with methylfluorosulfonate in chloroform [88 - 95 % yield] and the water-soluble ammonium chlorides 6a - c were obtained in quantitative yield by ion exchange chromatography. The macrocycles 6a - c are hygroscopic and crystallize with stoichiometric amounts of water [4]. For comparison compound 7 was synthesized in a similar way.

The ^{1}H-NMR chemical shifts of 6a - c in D_2O were found to be strongly concentration dependent due to aggregation. A plot of the ^{1}H-chemical shifts versus the concentration for 6c (Figure 1) shows marked upfield shifts with increasing concentration for hydrogen atoms close to the quarternary ammonium nitrogens, whereas the hydrogens of the methylene bridges and the aromatic protons 8-H, 12-H, surrounding the cavity, give chemical shift values passing through a minimum [for [6c]: $(6 \pm 1) \cdot 10^{-3}$ M]. The change of the chemical shifts with increasing concentration is accompanied by strong line broadening of all signals. The nature of this aggregation will be the subject of further investigations.

From the chemical shift data, the critical micelle concentration (CMC) of 6c was derived to be $(2.6 \pm 0.8) \cdot 10^{-4}$ M. Independently, the CMC was determined by light scattering [5]: aqueous solutions of 6c showed a strong increase of the relative stray light intensity at concentrations higher than $(2.5 \pm 0.2) \cdot 10^{-4}$ M.





6c

Figure 1: ^{1}H-NMR chemical shifts as a function of the concentration of 6c in D_2O at 303 K (TSP as external standard).

To exclude the interference of aggregation effects, all investigations of host-guest-complexation were carried out in concentration ranges below the CMC of the host compound. Clear spectroscopic evidence for 1:1 inclusion complex formation due to strong hydrophobic binding was obtained for 6c as a host molecule and 2-p-toluidinylnaphthalene-6-sulfonate (TNS) as a fluorescent hydrophobic guest (Figure 2). TNS, which exhibits a very weak fluorescence in water (Φ = 0.008) but strong fluorescence in a less polar environment (e.g. $\Phi_{Ethanol}$ = 0.52) [6] shows a pronounced fluorescence enhancement in aqueous solution in the presence of 6c, whereas a much smaller intensity increase is found in the presence of 6a, 6b and 7. We explain these spectral data by the different cavity size of 6a - c and the lack of a nonpolar binding cavity in 7: only the cavity of 6c is large enough to completely enclose the naphthalenesulfonate residue of TNS; the cavities of 6a and 6b are presumably too small for effective accommodation of the naphthalene moiety. Beside the strong increase in fluorescence quantum yield, the considerable shift from λ = 500 nm for TNS in water to λ = 445 nm in the presence of 6c provides another evidence that the cavity represents a good hydrophobic binding site.

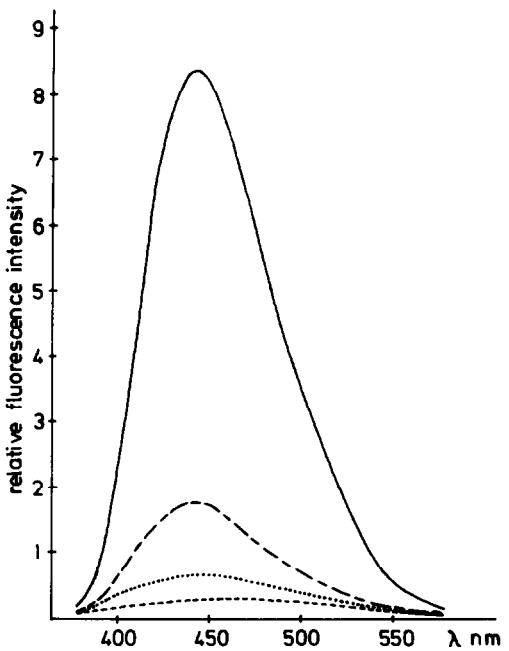


Figure 2: Fluorescence spectrum of $3.6 \cdot 10^{-6}$ M TNS in water upon addition of $1 \cdot 10^{-4}$ M 6c (———), 6b (— — —), 6a (·····) and 7 (------). The excitation wavelength is 360 nm.

The Benesi-Hildebrand plot [7] of the fluorescence intensity gave a straight line, indicating that 6c and TNS form a 1:1 complex in the considered concentration range [8]. The association constant could be calculated to be $K_{ass} = (4.3 \pm 0.2) \cdot 10^3\ M^{-1}$ and is in the same range as K_{ass} for the 1:1 complex between ß-cyclodextrin and TNS ($K_{ass} = 1.5 \cdot 10^3\ M^{-1}$) [9].

2,7-Dihydroxynaphthalene (8), for which large upfield shifts in the ^{1}H-NMR spectrum had been reported due to inclusion complex formation with a water-soluble paracyclophane [2c], was chosen as guest molecule in ^{1}H-NMR studies. The association constant for the 1:1 inclusion complex 6c - 8 was determined by competitive inhibition using TNS as fluorescent probe, and K_{ass} was found to be $(1.2 \pm 0.1) \cdot 10^3\ M^{-1}$ [10]. Table 1 shows the ^{1}H-NMR chemical shift changes for the protons of 8 in the presence of 6c at different concentrations. Below the CMC of 6c [11] only a weak upfield shift was observed, whereas with increasing aggregation of 6c the upfield shifts became more and more important and line broadening appeared. The chemical shift changes for 1-H and 4-H of 8 were much stronger than the one for 3-H. The host mole-

cule 6c showed only very small upfield shifts ($\Delta\delta$ = 0.1 ppm at most).

concentration (M)		$\Delta\delta$(ppm) 8		
[6c]	[8]	1-H	3-H	4-H
$1\cdot10^{-4}$	$5\cdot10^{-5}$	+0.07	+0.02	+0.07
$2\cdot10^{-4}$	$1\cdot10^{-4}$	+0.13	+0.05	+0.12
$8.1\cdot10^{-4}$	$4\cdot10^{-4}$	+0.30	+0.13	+0.28
$5.1\cdot10^{-3}$	$2.55\cdot10^{-3}$	+0.54	+0.20	+0.54

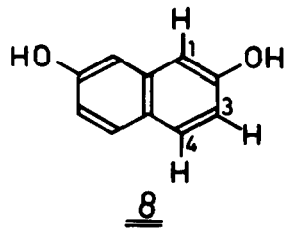


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Table 1: Chemical shift changes ($\Delta\delta$) of 8 in the presence of 6c at various concentrations in D_2O, TSP as external standard, T = 303 K; $\Delta\delta$(ppm) = δ 8(D_2O) - δ 8(D_2O + 6c).

As the chemical shift changes due to aggregation (Figure 1 and Table 1) are more important than the changes observed for 1:1 inclusion complex formation, ^{1}H-NMR spectroscopy in our hands was found less suitable than fluorescence spectroscopy for monitoring 1:1 complex formation. We feel that the association behaviour of the host molecule in the presence of the guest molecule should always be studied before assigning ^{1}H-NMR shifts to the formation of a 1:1 inclusion complex.

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References and footnotes

1) W.P.Jencks, "Catalysis in Chemistry and Enzymology", Chapter 8, McGraw-Hill,New York (1969).

2) (a) Y.Murakami, A.Nakano, K.Akiyoshi and K.Fukuya, J.Chem.Soc.,Perkin Trans.1 1981, 2800; (b) I.Tabushi, Y.Kimura and K.Yamamura, J.Am.Chem.Soc. 103, 6486 (1981); (c) K.Odashima, T.Soga and K.Koga, Tetrahedron Lett. 22, 5311 (1981); (d) S.P.Adams and H.W.Whitlock, J.Am.Chem.Soc. 104, 1602 (1982); and references cited in (2a) - (2d).

3) All new compounds gave satisfactory ir, ^{1}H-NMR, mass spectral and elemental analysis data.

4) The amount of water in the crystals of 6a - c was determined by elemental analysis and did not change by exposing the crystals to the air over the period of a week.

5) Light scattering at 90° was monitored with a SLM 8000 spectrofluorometer at λ=375nm;T=293K.

6) W.O.McClure and G.M.Edelman, Biochemistry 5, 1908 (1966).

7) H.A.Benesi and J.H.Hildebrand, J.Am.Chem.Soc. 71, 2703 (1949).

8) The concentrations in water were [TNS]=$2.24\cdot10^{-6}$ M, [6c] = $1.01\text{-}10.1\cdot10^{-5}$ M; T=293 ± 0.1 K; the excitation wavelength was 360 nm, the fluorescence intensity was measured at 445 nm with a SLM 8000 spectrofluorometer.

9) H.Kondo, H.Nakatani and K.Hiromi, J.Biochem. 79, 393 (1976).

10) T=293 ± 0.1 K; excitation wavelength=360 nm, fluorescence intensity detected at 445 nm, [TNS]=$2.09\cdot10^{-6}$ M, [6c] = $1.01\text{-}10.1\cdot10^{-5}$ M, [8] = $1.3\cdot10^{-3}$ M; for the method see: K.Mochida, A.Kagita, Y.Matsui and Y.Date, Bull.chem.Soc.Jpn. 46, 3703 (1973).

11) Light scattering showed that the CMC of 6c in water was not significantly affected by the presence of $1\cdot10^{-4}$ M 2,7-Dihydroxynaphthalene.

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