Piperidine aminoxyl radicals as EPR probes for exploring the cavity of a water-soluble cryptophane

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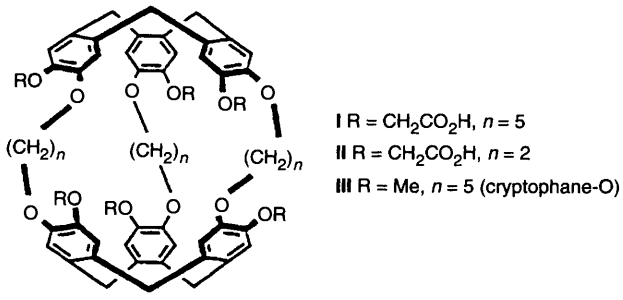
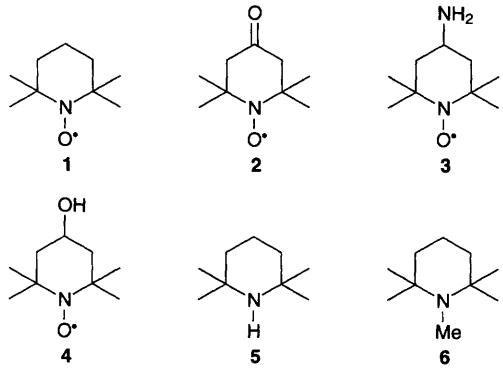
Piperidine aminoxyl radicals are reversibly bound in the cavity of a water-soluble cryptophane with an affinity of the order of 10^3 dm^3 mol^{-1}; the complexation equilibrium is slow on the EPR timescale and the magnitude of the nitrogen hyperfine splittings of the bound substrates indicates that the cavity of this cryptophane has a polarity comparable to that of halogenated solvents.

The cryptophanes[1] are hollow molecules made of two cyclotriveratrylene caps attached to one another by three bridges (Scheme 1). Their cavity is able to accommodate neutral guests, such as methane[2] or halogenoalkanes,[3] as well as soft cationic species such as R_4N^+ or acetylcholine.[4] In order to better understand some of the factors governing the formation and the stability of such complexes, it is of interest to explore the interior of these hosts, and particularly to characterize the polarity of the inner phase. Along these lines, we report here a series of EPR experiments on the interactions of cryptophane **I** with the piperidine aminoxyl radicals **1–4**† in water, which lead to the reversible formation of well characterized intramolecular inclusion complexes.[5,6]

The hexaacid cryptophane **I** was synthesized from cryptophane-O **III** as previously described.[4] A stirred suspension of **I** in water was titrated by addition of 1 mol dm^{-3} NaOH until four of the six acid groups were ionized to give a 10^{-3} mol dm^{-3} solution (pH 7.0 ± 0.5). Samples suitable for the EPR measurements were prepared by adding 20 μl of a water solution of known concentration (10^{-4}–10^{-3} mol dm^{-3}) of the aminoxyl radical to 20 μl of the 10^{-3} mol dm^{-3} solution of cryptophane **I**.‡





Scheme 1

When cryptophane **I** was added to a solution of **1** in water, the high-field line in the EPR spectrum split into two components which we assign to free and bound **1**, respectively, Fig. 1(*a*,*b*). The changes of the EPR spectrum were even larger when host **I** was added to a solution of **2**, in which case each line was split into two components, and the spectra of the free and bound species were totally resolved, Fig. 1(*c*,*d*). Similar phenomena were observed with **3** and **4** as the substrates. These spectra indicate the presence of free and complexed species exchanging slowly on the EPR timescale.

In order to confirm that the observed spectral changes were due to the inclusion of **1–4** in the cavity of **I**, rather than to interactions of these radicals with the external surface of the

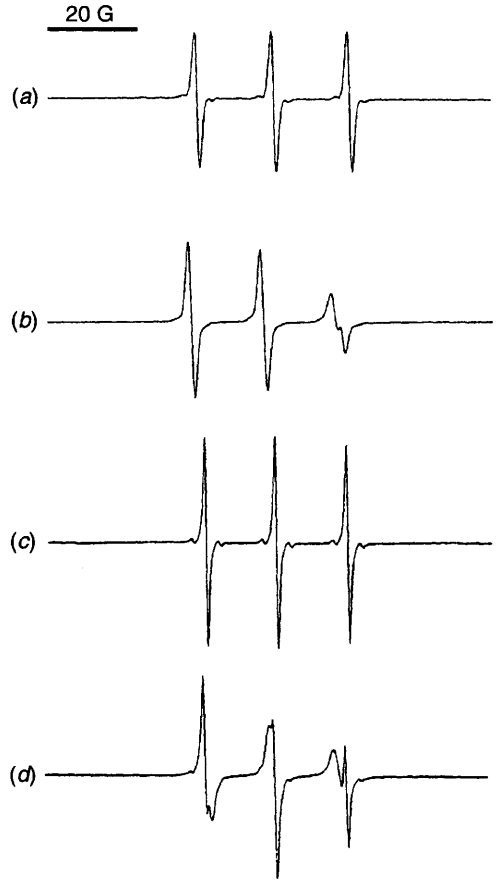


Fig. 1 X-Band EPR spectra at 293 K in water (*a*) of 5 × 10^{-4} mol dm^{-3} Tempo **1** alone and (*b*) in the presence of 5 × 10^{-4} mol dm^{-3} cryptophane **I**; (*c*) of 5 × 10^{-4} mol dm^{-3} Tempone **2** alone and (*d*) in the presence of 5 × 10^{-4} mol dm^{-3} cryptophane **I** (spectral width = 100 G)

Table 1 Nitrogen hyperfine splittings (Nhfs) of inclusion complexes of cryptophane **I** and aminoxyl radicals **1–4** at 293 K

Aminoxyl radical	Nhfs (G) in Pentane	Toluene	CH_2Cl_2	$CHCl_3$	Ethanol	Water	Cryptophane
1	15.3	15.5	15.9	16.1	16.2	17.3	15.9
2	13.5	14.2	14.5	14.8	15.0	16.0	14.6
3	14.7	15.3	15.7	15.9	16.2	16.8	15.4
4	14.7	15.4	15.7	15.8	16.1	17.2	15.8

host, the same experiments were carried out using cryptophane **II**, the cavity of which is too small to accommodate these bulky piperidine derivatives. Addition of **II** to an aqueous solution of **1–4** had no detectable effect on their EPR spectra. Further evidence that the cavity of cryptophane **I** is large enough to contain species of the size of aminoxyl radicals **1–4** (*ca.* 200–220 Å^3) was obtained from ^{1}H NMR studies of the interaction of this host with piperidine derivatives **5** and **6**; these guests proved to be reversibly complexed by **I** in water, with slow exchange being observed at room temperature between the free and complexed species.§

The complexed guests showed distinctly smaller nitrogen hyperfine splittings (Nhfs) than the corresponding free species (Table 1). This effect is expected since the environment of the N–O· group within the cryptophane cavity is certainly less polar than in bulk water, and it is known that the Nhfs decrease as the solvent polarity decreases.[7] This observation provided additional evidence that the aminoxyl substrates are lodged in the host cavity, where they are protected from the surrounding water. Comparison of the magnitude of the Nhfs of the complexed radicals with those measured in different solvents ranging from pentane to water suggests that, in spite of its aromatic structure, the interior of this cryptophane has in fact the polarity of bulk dichloromethane or chloroform.

It proved difficult to obtain reliable binding constants through simulation of the EPR spectra themselves. Values scattered in the range 10^3 to 2×10^4 dm^3 mol^{-1} were obtained for the **1** ⊂ **I** complex, and this is perhaps a limitation of the method. Nevertheless, competition experiments carried out in the prescence of 1 equiv. of Me_3N^+Et iodide (K_a = *ca.* 3200 dm^3 mol^{-1})[4] and which showed a partial displacement of the bound aminoxyl radicals allowed their affinities to be ranked in the order **1** > **2** ≈ **3** > **4**, and to estimate the stability of the **1** ⊂ **I** complex at 1000–2000 dm^3 mol^{-1}. A two-fold excess of a stronger competitor such as choline (K_a *ca.* 5000 dm^3 mol^{-1}) totally displaced **1–4** from the host cavity to the aqueous phase, whereas a weak competitor such as Me_3NH^+ (K_a = *ca.* 5 dm^3 mol^{-1}) had no detectable effect. The lower affinities of **2–4** *vs.* **1** is certainly due to the presence at C-4 of an oxo, amino or hydroxy group, which increases their hydrophilic character. Compounds **1–4** are the largest molecules that have been enclosed in the cavity of cryptophane **I** thus far. As these substrates are neutral species, hydrophobic effects, desolvation of the host, and the relative size and shape of the partners, which determine the strength of van der Waals interactions, certainly provide major contributions to the stabilities of the complexes.

The relative dynamics of the free and bound substrate is also an important parameter, because of its implication in the entropy of complexation.[4] We sought to estimate the degree of freedom of **1–4** in the host cavity by measuring their rotational correlation times τ_C from the EPR spectra.[8] The values of τ_C for the complexed species were thus found to be 16- to 20-fold greater than those of the free species (Table 2). However, interpretation of this slow motion in the cavity is not straightforward and must await the determination of the correlation time for the reorientation of the cryptophane itself.

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Table 2 Rotational correlation time τ_c of aminoxyl radicals in water (reference sample) and in an aqueous solution of cryptophane **I**

Aminoxyl radical	τ_c (10^{-11} s) Reference sample	Cryptophane **I**	τ_c (bound)/τ_c (free)
1	2.5	50.5	20.2
2	2.2	39.5	17.9
3	3.5	56.8	16.2
4	3.3	65.0	19.7

Footnotes

† Aminoxyl radicals **1–4** were purchased from Aldrich and Molecular Probes and used without further purification; [2,2,6,6-tetramethylpiperidine-1-oxyl **1** (TEMPO); 4-oxo-2,2,6,6-tetramethylpiperidine-1-oxyl **2** (TEMPONE); 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl **3** (TEMPAMINE) and 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl **4** (TEMPOL)]. In CPK models the size and shape of these substrates match closely the cavity of cryptophane **I**.

‡ EPR spectra were recorded with a Bruker ESP 380E spectrometer. The field modulation amplitude was 1 G and the incident microwave power was lower than 5 mW for the EPR measurements. The temperature was controlled by a Bruker variable-temperature unit. The sample solutions were filled in 1 mm (o.d.) quartz tubes and were not degassed.

§ These experiments were carried out at 200 MHz in D_2O–NaOD (pH ≫ 7); the binding constants of the (**5** ⊂ **I**) and (**6** ⊂ **I**) complexes were not determined.

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