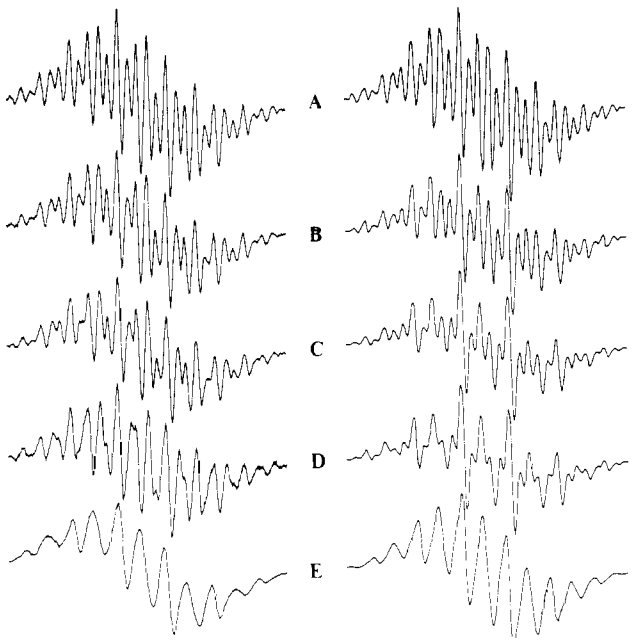

Figure 1. Experimental (left) and simulated (right) EPR spectra of the highest field multiplet of $(t\text{-Bu})_2C{=}NO\cdot$ in isopentane: (A) $-50°$, $k = 0$; (B) $-37°$, $k = 2.1 \times 10^5$ sec^{-1}; (C) $-27°$, $k = 4.2 \times 10^5$ sec^{-1}; (D) $14°$, $k = 6.3 \times 10^5$ sec^{-1}; (E) $72°$, $k = 1.3 \times 10^6$ sec^{-1}.

Unfortunately, the lifetimes of most aryliminoxys appear to be too short[17] to permit their study by NMR.

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Nitroxides. LXX. Electron Spin Resonance Study of Cyclodextrin Inclusion Compounds

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Abstract: Complexation of nitroxide radicals with cyclodextrins has been studied by electron spin resonance spectroscopy (esr). There is evidence for a complexation equilibrium between 2,2,6,6-tetramethylpiperidine-1-oxy (**1**) and β-cyclodextrin, but not with α-cyclodextrin. Substituted tetramethylpiperidine-*N*-oxy radicals **2** and **3** are not complexed by α- or β-cyclodextrins. On the other hand, oxazolidine-*N*-oxy derivatives are complexed either by α- and β-cyclodextrins or by β-cyclodextrin only, depending on the size of the radicals. Hyperfine splittings are consistent with a nonpolar environment of the complexed nitroxide group. These results are interpreted as evidence for inclusion of the nitroxide radical in the cyclodextrin cavity. This is in agreement with an independent ultraviolet spectroscopy study on 5-nitro-1,1,3,3-tetraethylisoindoline-2-oxy (**9**). Different models of inclusion are discussed. Quantitative information on cyclodextrin–guest equilibrium can easily be obtained using the biradical **10** [dispiro(2,2,6,6-tetramethylpiperidine-1-oxy)-4,4′-(oxazolidine-3′-oxy)-2′,1″-cyclohexane] for which the following thermodynamic data on its association equilibrium with β-cyclodextrin can be measured: $\Delta H_{assoc} = -12 \pm 2$ kcal/mol; $\Delta S_{assoc} = -30 \pm 3$ cal/(deg mol).

Cyclodextrins are known to give noncovalent inclusion complexes with various organic molecules in aqueous solution.[1,2] For this reason, they have been used as models for enzymes[3,4] or proteins.[5]

The structure of cyclodextrins has been established by chemical studies.[6-8] The structures of some cyclodextrin inclusion complexes have been determined by X-ray diffraction:[9-11] α-cyclodextrin (β-cyclodextrin) is a cyclic molecule consisting of six (seven) α −1 → 4 linked D-glucopyranose units. The principal difference between α- and β-

cyclodextrins is the size of the cavity in the center of the molecule. The diameter of the "hole" is *ca.* 6 Å in α-cyclodextrin and 7.5 Å in β-cyclodextrin.[5]

Solutions of cyclodextrin inclusion compounds have been studied by nuclear magnetic resonance (nmr)[12] and ultraviolet and visible spectroscopy (uv).[13] In addition, fluorescence[14] and circular dichroism measurements[15] have been made. These techniques have given evidence for inclusion of aromatic compounds[13,16] and nucleic acids.[17] These results[13] can be ascribed to an equilibrium between cyclodextrin (C), guest molecule (G), and cyclodextrin-guest complex (CG) in solution:

$$C + G \rightleftharpoons CG$$

For instance, the uv spectral change observed for *p-tert*-butylphenol in the presence of α-cyclodextrin[13] is attributed to an inclusion equilibrium, the guest molecule being inside the cyclodextrin cavity in a medium of polarity close to that of dioxane. It has been shown by the same technique that I_3^- ions give inclusion compounds with cyclodextrins.[18]

Thermodynamic constants of cyclodextrin-guest equilibria have been determined using vapor-phase chromatography for hydrocarbon guests[19-21] and by calorimetry for various other guest compounds.[22]

Electron spin resonance spectroscopy (esr) has also been used. Paton and Kaiser have shown that rotational correlation time τ_C in solution is greater when a nitroxide radical is covalently bonded to β-cyclodextrin than when noncovalently associated.[23a] Precipitates obtained from spin-labeled fatty acids and cyclodextrins have been studied by Griffith and coworkers. It has been shown that "included" radicals have an anisotropic motion at room temperature[24,25] However, these two examples are not the only possible applications of esr. Detailed information can be obtained on complexation equilibria, radical anisotropic motion, and polarity of its surroundings. We shall examine two possibilities. First, for an isolated nitroxide in solution a three-line esr spectrum is observed. It is due to hyperfine interaction with the nitrogen nucleus. The isotropic hyperfine coupling constant, a_N, and the Landé g factor are sensitive functions of the polarity of the medium surrounding the nitroxide group.[26-33] In such a case, the rotational correlation time τ_C can be measured from the esr line width. Qualitatively, when the high-field line is narrow, τ_C is small ($\sim 10^{-11}$ sec); when this line broadens, τ_C increases. In a more quantitative manner, the rotational correlation time for the motion of the paramagnetic molecule can be obtained from formula

$$\tau_C = A\Delta H_{(m=+1)}[(I_{m=+1}/I_{m=-1})^{1/2} - 1] \qquad (1)$$

where $\Delta H_{(m=+1)}$ is the peak-to-peak line width in gauss of the derivative of the low-field absorption line (corresponding to the nuclear magnetic moment $m = +1$). $I_{(m=+1)}$ and $I_{(m=-1)}$ are the corresponding peak-to-peak heights for the low- and high-field lines, respectively. Formula 1[34a,b] is deduced from Kivelson's theory[34c] following a method already published.[34d] The constant A can be calculated from the principal values of **g** and **a** tensors of the nitroxide radical:

$$A = 15\pi(3)^{1/2}g\beta/8hH_0b\Delta\gamma$$

where $b = 4\pi/3(a_\parallel - a_\perp)$, $\Delta\gamma = -\beta/\hbar(g_\parallel - g_\perp)$, and H_0 is the applied field in gauss. We have calculated $A = 6.6 \times 10^{-10}$ using published data for di-*tert*-butyl nitroxide.[35] We shall use this value for all radicals studied, because it does not change much between various radicals.

A second possibility will be obtained for an equilibrium between nitroxide radical and cyclodextrin (C) in aqueous solution. Two paramagnetic species exist: uncomplexed radical (R) and the cyclodextrin radical complex (CR). If we

$$C + R \rightleftharpoons CR$$

accept that the rotational correlation time increases with the volume of the paramagnetic species,[36] the correlation time of the complexed radical must be larger than the correlation time of the unbound nitroxide in aqueous solution. Qualitatively, an increase of the esr high-field line width will be evidence for a complexation. Two cases must be distinguished for a more quantitative discussion. If the cyclodextrin nitroxide equilibrium is "slow" (relative to esr characteristic frequencies), the esr spectrum will be the superposition of the free and complexed nitroxide spectra, and the resulting spectrum will or will not be resolved. If the equilibrium is "fast," the resulting spectrum will be the weighted average of the two spectra. In both cases, the resulting spectrum depends on the respective concentration of free and bound species, on their respective hyperfine coupling constant, g factors, and line widths, and on the rate of exchange of radical between aqueous solution and cyclodextrin. We did not try to obtain these values by spectral simulation. For the discussion of our results, we shall use the following simplification: if the resulting spectrum is not "visually" resolved into two spectra, peak-to-peak line width $\Delta H_{(m=+1)}$, line heights ($I_{m=+1}$, $I_{m=-1}$), and the separation δ_1,δ_2 between the three lines can be measured on this spectrum. From these measured values, the apparent hyperfine interaction (a^*), apparent Landé factor (g^*), and apparent rotational correlation time (τ_C^*) will be defined as if the observed spectrum resulted from a single species. The equilibrium between nitroxide and cyclodextrin is qualitatively characterized by these apparent values.

Experimental Section

We have studied esr spectra of various nitroxide radicals (Figure 1) in the presence of α- and β-cyclodextrins solutions. α- and β-cyclodextrins were obtained from Pierce Chemical Co. and used without further purification.

Esr measurements were made using a Varian V-4502 spectrometer. Uv spectra were recorded on a Beckman DK-2A instrument. Except where specified differently, aqueous α- and β-cyclodextrin solutions have always been taken as 10^{-3} M and total nitroxide concentration as 3×10^{-5} M. Samples were prepared by adding 10 μl of a $3 \times 10^{-}$ M solution of radical in water (w) or in ethanol (e) to 1 ml of water (reference sample) or to 1 ml of cyclodextrin solutions.

Results

(a) Tetramethylpiperidine-*N*-oxy Derivatives. The three spectra obtained for 2,2,6,6-tetramethylpiperidine-1-oxy (**1**)[31,37,38] added to pure water or to water–α- or β-cyclodextrins solutions are shown in Figure 2.[49]

Compared with the reference sample, there is only a broadening of the high-field line for β-cyclodextrin. Table I shows the results obtained for radical **1**, for 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxy (**2**),[31] and for 4-oxo-2,2,6,6-tetramethylpiperidine-1-oxy (**3**).[31,38] An increase in the apparent rotational correlation time τ_C^* is observed only for radical **1** in the β-cyclodextrin solution. For radicals **2** and **3**, the spectra in presence of α- and β-cyclodextrins are identical with that of the reference sample.

These results can be interpreted as evidence for an association between **1** and β-cyclodextrin. There is no detectable association between **1** and α-cyclodextrin, nor between **2, 3,** and α- or β-cyclodextrins at 3×10^{-5} M radical concentration in 10^{-3} M cycloamylose concentration. However, at higher cycloamylose concentration, complexation has been observed by Kaiser and Flohr.[23b]

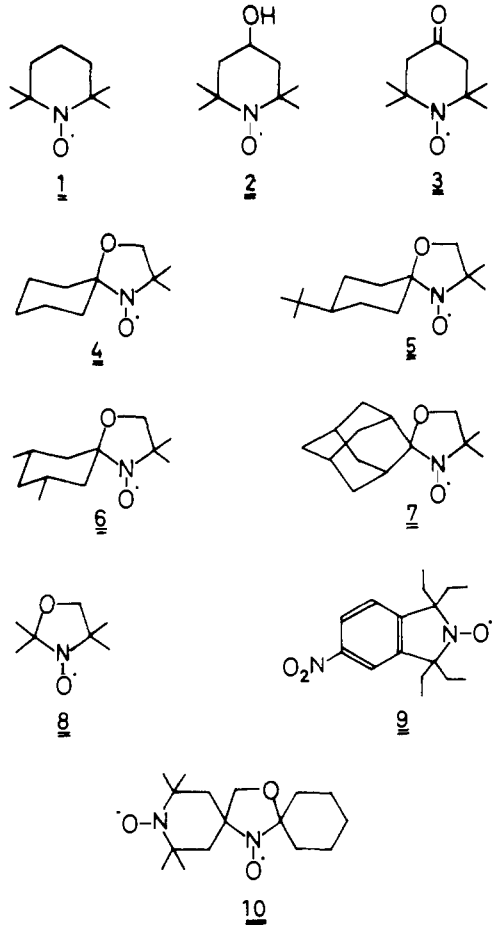

Figure 1

(b) **Oxazolidine-*N*-oxy Derivatives.** Table II gives the results for a series of substituted oxazolidine-oxy radicals in ethanol solution: spiro(1,2′-cyclohexane(4′,4′-dimethyloxazolidine-3′-oxy)) (**4**),[39] spiro(1,2′-(4-*tert*-butylcyclohexane)-(4′,4′-dimethyloxazolidine-3′-oxy)) (**5**),[40] spiro(1,2′-(3,5-dimethylcyclohexane)-(4′,4′-dimethyloxazolidine-3′-oxy)) (**6**),[40] spiro(2,2′-adamantane(4′,4′-dimethyloxazolidine-3′-oxy)) (**7**),[40] 2,2,4,4-tetramethyloxazolidine-3-oxy-(**8**).[41]

A broadening of the high-field line relative to the reference sample is observed for **4, 5,** and **7** in the presence of both α- and β-cyclodextrins and for **6** in the presence of β-cyclodextrin only. This is reflected by the increase of apparent rotational correlation time. When the concentration of radical **5** is increased, the high-field line broadens. These changes are reversible; when a concentrated solution of radical **5** is diluted, the high-field line narrows to its original width (Table II).

For radical **8,** there is no line broadening in α-cyclodextrin compared with the reference sample, but in β-cyclodextrin, a different spectrum (Figure 3) is observed; the high-field line shows two components. These two lines are attributed to free and complexed radical. In this case, the spectra of the free and bound species are sufficiently different so that the superposition of the two spectra can be distinguished. This is a clear evidence for a "slow" exchange of radical between aqueous solution and cyclodextrin.

(c) **Radical Surrounding.** Table III gives separation δ_1 and δ_2 between the three hyperfine lines of the radicals forming complexes with α- or β-cyclodextrin. δ_1 and δ_2 are not always equal. This is probably due to second-order effects.[53] For radical **1,** a small decrease on δ_1 and δ_2 (*ca.* 0.1 G) between reference and β-cyclodextrin solutions is observed. For radicals **4, 5, 6,** and **7,** the decrease between reference and α- or β-cyclodextrin is larger (*ca.* 0.3 G). As shown in the case of radical **5,** this separation is concentration dependent; δ_1 and δ_2 decrease when the concentration in radical increases. For radical **8,** the esr lines are so narrow as to allow the observation of two species, one having the same nitrogen splitting as the reference sample, and the second having a nitrogen splitting 0.6 G smaller.

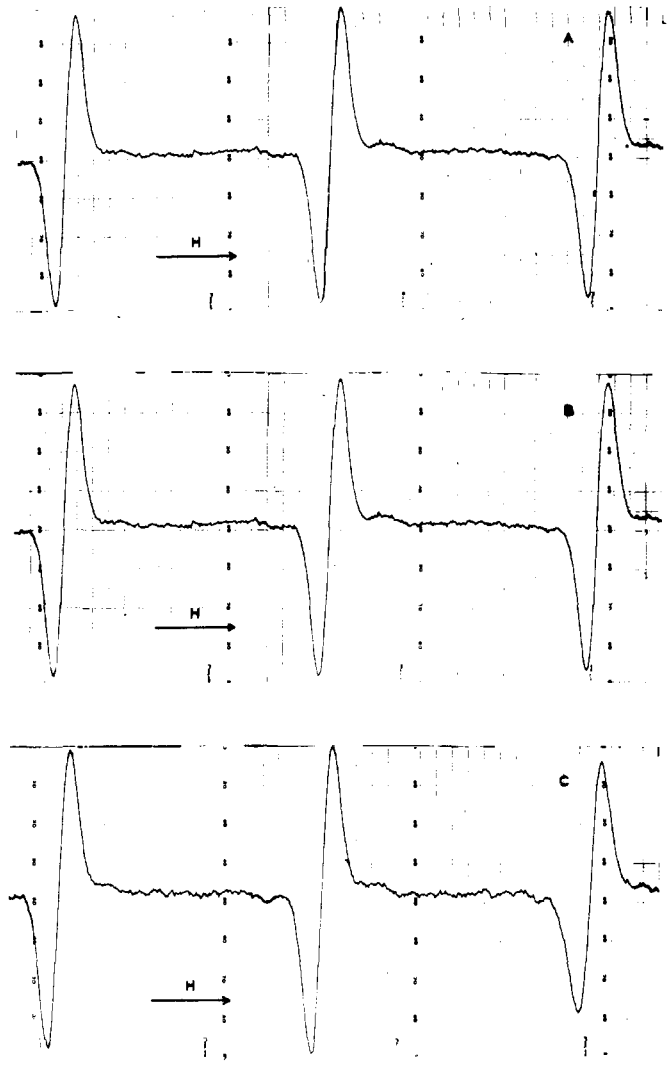

Figure 2. Esr spectrum of **1** (3×10^{-5} *M*) at 25°: (A) in water solution, (B) in α-cyclodextrin solution (10^{-3} *M*), and (C) in β-cyclodextrin solution (10^{-3} *M*). The arrows in the figure cover 5 G.

(d) **Equilibrium Displacement by a Diamagnetic Molecule.** This esr study of the complexation between cyclodextrin and nitroxide radical may also provide some information on complexation equilibrium between a diamagnetic molecule and cyclodextrin. For instance, addition of I_3^- to an aqueous solution of radical **1** with β-cyclodextrin or radical **7** with α-cyclodextrin modifies the esr spectrum: the high-field line narrows; the apparent correlation time decreases. A similar addition to the reference sample does not modify its spectrum (Table IV). This suggests that quantitative knowledge of the equilibrium between cyclodextrin and a nitroxide radical may be used to afford quantitative data on the equilibrium between cyclodextrin and diamagnetic molecules. Similar displacement techniques have been used.[42,43]

(e) **Evidence for Inclusion of Nitroxide in Cyclodextrins.**

Table I

Compd	τ_C*, sec[a] In reference sample	In α-cyclodextrin solution	In β-cyclodextrin solution
1	$<10^{-11}$	$<10^{-11}$	9.0×10^{-11}
2	$<10^{-11}$	$<10^{-11}$	$<10^{-11}$
3	1.5×10^{-11}	1.5×10^{-11}	1.5×10^{-11}

[a] The precision of the measurements of parameters from the spectra do not permit the calculation of $\tau_C^* < 10^{-11}$ sec.

Table II

Compd	τ_C*, sec In reference sample	In α-cyclodextrin solution	In β-cyclodextrin solution
4	5.0×10^{-11}	1.0×10^{-10}	1.7×10^{-10}
5 ($c = 3 \times 10^{-5}$ M)	$<10^{-11}$	4.2×10^{-11}	6.0×10^{-11}
5 ($c = 3 \times 10^{-4}$ M)	$<10^{-11}$	...[a]	9.0×10^{-11}
6	$<10^{-11}$	$<10^{-11}$	1.4×10^{-10}
7	$<10^{-11}$	8.8×10^{-11}	1.5×10^{-10}

[a] The τ_C* could not be determined because of the presence of a precipitate.

Table III

Compd	δ, G[a] In reference sample δ_1	δ_2	In α-cyclodextrin solution δ_1	δ_2	In β-cyclodextrin solution δ_1	δ_2
1	17.2	17.3	17.2	17.3	17.1	17.2
2	17.0	17.1	17.0	17.1	17.0	17.1
3	16.0	16.2	16.0	16.2	16.0	16.2
4	15.9	15.9	15.7	15.7	15.4	15.6
5 ($c = 3 \times 10^{-5}$ M)	15.9	15.9	15.6	15.6	15.5	15.6
5 ($c = 3 \times 10^{-4}$ M)	15.9	15.9			15.3	15.4
6	15.9	15.9	15.8	15.9	15.4	15.6
7	15.6	15.6	15.4	15.4	15.1	15.3
8	15.9	15.9	15.9	15.9	15.9	15.9[b] 15.3[c]

[a] δ_1 and δ_2 are the distances between respectively the lines corresponding to the nuclear magnetic moment, $m = +1$, $m = 0$ and $m = 0$, $m = -1$. [b] In water. [c] Included in cyclodextrin.

Table IV

Compd	τ_C*, sec	
1	In β-cyclodextrin solution 9.0×10^{-11}	In β-cyclodextrin solution + I_3^- 5.3×10^{-11}
7	In α-cyclodextrin solution 8.8×10^{-11}	In α-cyclodextrin solution + I_3^- 5.1×10^{-11}

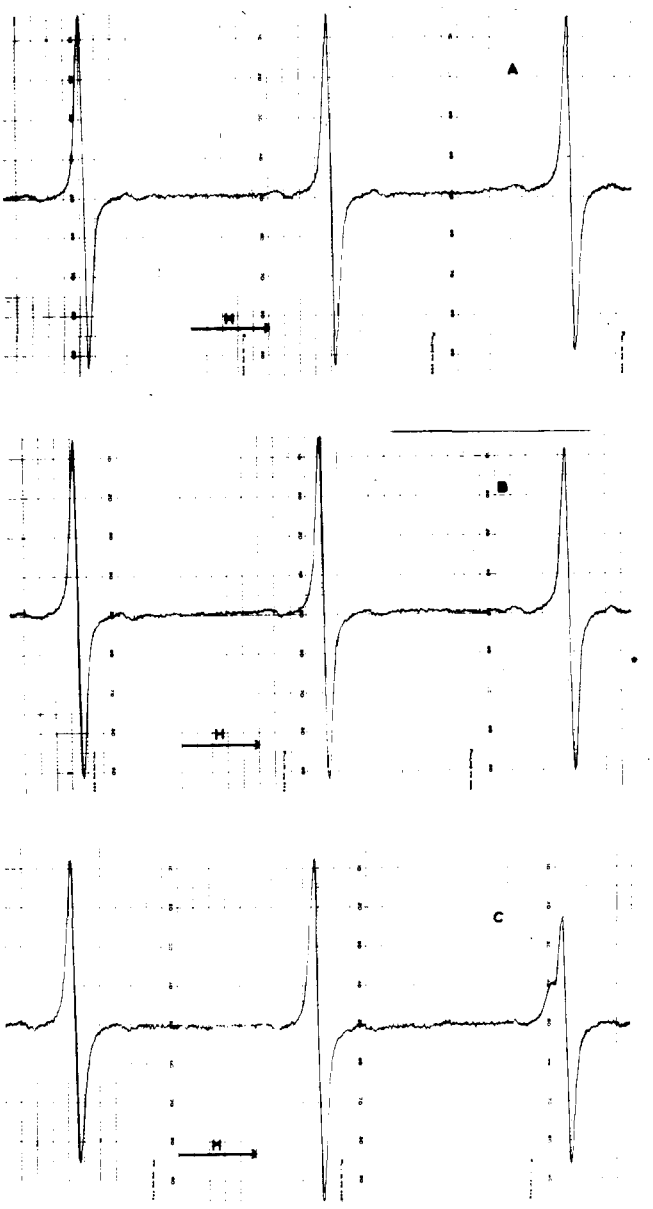

Figure 3. Esr spectrum of **8** (3×10^{-5} M) at 25°: (A) in water solution, (B) in α-cyclodextrin solution (10^{-3} M), (C) in β-cyclodextrin solution (10^{-3} M). The arrows in the figure cover 5 G.

The previous results are in agreement with a complexation equilibrium. It is well known that cyclodextrins form inclusion complexes with diamagnetic molecules. Esr results can be interpreted as evidence for inclusion of the nitroxides. The fact that the apparent hyperfine coupling a_N* of the complexed radical is smaller than the hyperfine splitting of the reference sample is evidence that the complexed radical is surrounded by groups less polar than water. It has been shown that the cavity of the cyclodextrin host fulfills this requirement.[13] Inspection of apparent correlation times τ_C* shows that radicals **1, 6,** and **8** form complexes with β-cyclodextrin only. Since α- and β-cyclodextrins differ only in the sizes of their cavities, surface adsorption of the nitroxides on the outside of the cavity can reasonably be excluded. Furthermore, such an inclusion is consistent with examinations of space-filling molecular models. Examination of such molecular models reveals various possibilities for inclusion. The orientation of the nitroxide relative to the cyclodextrin cylinder is defined by two angular parameters. For monocyclic radicals, two limiting cases A or B are shown in Figure 4. For bicyclic radicals, the situation will be similar to that of a monocyclic radical if the nitroxide-containing ring is included in the cavity (Figure 4, A and B), while the situation can be represented by Figure 4, case C, if the other ring is included in the cavity.

Table V shows the results from considerations on α- and β-cyclodextrin models and models of the various radicals (**1** → **8**). For instance, for radical **1** in case A, β + α − means that its model can be included with its NO group parallel to the axis of cyclodextrin cylinder, in β-cyclodextrin model and not in α-cyclodextrin model.

These considerations on models are consistent with the experimental results if radicals **1** and **8** have an A-type inclusion and radicals **4, 5, 6,** and **7,** a C-type inclusion. The difference between **1, 2,** and **3** may be related to polarity effects. It has already been shown that for similar steric factors, polar groups prevent inclusion.[13,44] This also applies to substituted nitroxides; complexation occurs preferentially

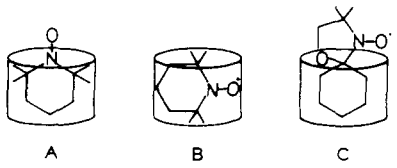

Figure 4. Limiting cases of inclusion.

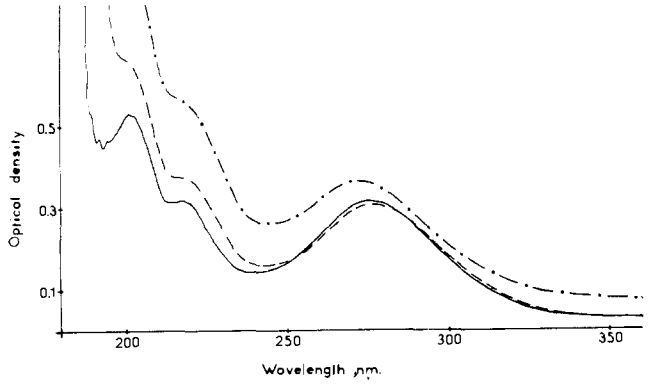

Figure 5. Ultraviolet absorption spectra of radical **9** (3×10^{-5} *M*) in (- - -) water solution, (——) α-cyclodextrin solution (10^{-2} *M*), and (—·—·) β-cyclodextrin solution (10^{-2} *M*).

with the nonpolar part of the molecule.

(f) Ultraviolet Study. We have also examined the complexation of a nitroxide radical by a technique different from esr. In the range of concentrations used, 5-nitro-1,1,3,3-tetraethylisoindoline-2-oxy (**9**)[45] can be studied both by esr and uv spectroscopy. It can be shown by esr that this radical forms an addition compound with β-cyclodextrin only (Table VI). In this case, however, no variation of the apparent hyperfine splitting relative to reference solution is observed; it must be concluded that the nitroxide group stays in a surrounding having water polarity. Uv spectra of various solutions (Figure 5) show that the 275-nm absorption band of radical **9** in water is not shifted in α-cyclodextrin solution but shifts to 270 nm in β-cyclodextrin solution. This band is known to undergo hypsochromic shift when solvent "polarity"[46] decreases.[47]

The β-cyclodextrin solution shift corresponds to a chromophore in a surrounding having dioxane polarity.[47] This indicates that in radical **9**, the nitroxide group is surrounded by water, while the aromatic group is inside the cyclodextrin cavity.[3,13]

(g) Use of a Biradical. In the case of the previous monoradicals, a quantitative determination of the free and complexed species may be difficult; *i.e.*, the spectral reconstitution depends on numerous parameters, and the inclusion-equilibrium thermodynamical-data determination will probably be more sensitive than the spectral reconstitution to the choice of these parameters. In order to be able to analyze our results in a simple quantitative way, we have used a nitroxide biradical.

It has been shown[48] that a biradical with large dipolar splitting can be used to study rotational correlation time τ_C between 10^{-11} and 10^{-8} sec. For $\tau_C \simeq 4 \times 10^{-11}$ sec, the esr spectrum displays an approximately 30-G wide single line. For 4×10^{-11} sec $< \tau_C < 10^{-8}$ sec, the line width increases continuously until a fully immobilized spectrum is obtained for $\tau_C \simeq 10^{-8}$ sec. The second derivative immobilized spectrum[48] shows four lines: two central lines separated by 250 G = D, and two external lines separated by *500 G = 2D*. We have studied complexation of biradical **10** [dispiro(2,2,6,6-tetramethylpiperidine-1-oxy) - 4,4′-(oxazolidine-3′-oxy)-2′,1″-cyclohexane][48] in the presence of β-cyclodextrin. When a 10^{-3} *M* solution of biradical **10** is added to a 5×10^{-2} *M* solution of β-cyclodextrin in a DMSO–water (1/1 by volume) mixture, a spectrum shown in Figure 6 is obtained. It consists of a 35-G wide central line, three narrow lines superposed on this central line, two external lines separated by 360 G, and two internal lines separated by 190 G. The three narrow lines are attributed to a small percentage (less than 3%) of monoradical, the broad line to the free biradical, and the four external lines to biradical complexed by β-cyclodextrin. Rotational correlation times can be determined from a calibration diagram:[48] $\tau_C = 1 \times 10^{-10}$ sec for free biradical and $\tau_C = 1.9 \times 10^{-9}$ sec for complexed biradical.

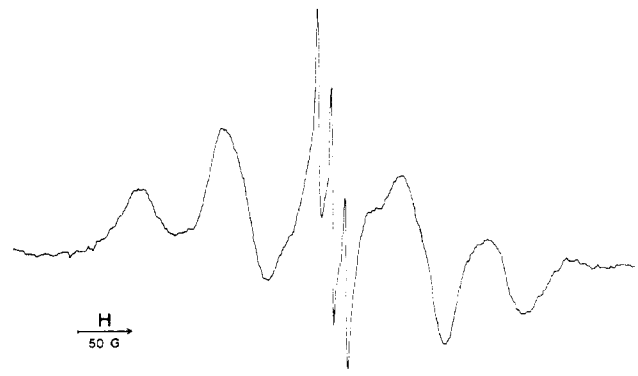

Figure 6. Esr spectrum of biradical **10** (10^{-3} *M*) in β-cyclodextrin solution (5×10^{-2} *M*).

Table V

Compd	Cases A	B	C
1	β + α −	β + α +	
2	β + α −	β + α −	
3	β + α −	β + α −	
4	β − α −	β + α +	β + α +
5	β − α −	β + α +	β + α +
6	β − α −	β + α +	β + α −
7	β − α −	β + α +	β + α +
8	β + α −	β + α +	β + α +

Table VI

	Compound 9 In reference sample	In α-cyclodextrin solution	In β-cyclodextrin solution
τ_C*, sec	4.2×10^{-11}	4.3×10^{-11}	9.7×10^{-11}
δ_1, G	15.1	15.1	15.1
δ_2, G	15.2	15.2	15.2

The association equilibrium constant is $K = (BC)/(B)(C)$, (B), (C), and (BC) being the free biradical, cyclodextrin, and complexed biradical concentrations. If (C_0) and (B_0) are respectively the total cyclodextrin and biradical concentrations, and if $(BC)^2$ is neglected, *K* can be written as:

$$K = (BC)/\{(B_0)(C_0) - (BC)[(C_0) + (B_0)]\}$$

where the only unknown is (BC). The equilibrium constant can be determined by knowing the concentration of the complexed biradical only. We calculate this quantity by comparison with an immobilized solution of known concentration. In order to obtain thermodynamic data for the complexation equilibrium, we have studied the temperature dependence of the esr spectra. We have verified that the complexed biradical esr line widths do not change with temperature. We note that in such a case the determination of con-

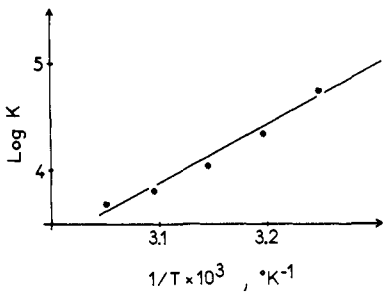

Figure 7. Variation of log K as a function of $1/T$ for biradical **10** in β-cyclodextrin solution.

centration by double integration of the esr spectra is equivalent to the measurement of the second-derivative spectrum peak height h. We shall write, $(BC) = kh$, where k is determined by comparison with a fully immobilized solution. Using this approximation, K is found to be 133 l. mol^{-1} at 308°K, 83.4 l. mol^{-1} at 313°K, 57.3 l. mol^{-1} at 318°K, 45 l. mol^{-1} at 323°K, and 39.6 l. mol^{-1} at 328°K. The errors are probably quite large. However, more accurate data can be given; when $\log K = \log kh - \log \{(B_0)(C_0) - kh[(C_0) + (B_0)]\}$ is plotted as a function of the reciprocal of absolute temperature, a graph (Figure 7) is obtained from which association enthalpy and entropy can be determined: $\Delta H_{assoc} = -12 \pm 2$ kcal/mol and $\Delta S_{assoc} = -30 \pm 3$ cal/(deg mol).

Similar values have been obtained for hexane and 2,3-dimethylbutane in α- and β-cyclodextrin derivatives.[19,20] Slightly smaller values for ΔH_{assoc} are usually found for other molecules.[2]

Conclusion

These results show that esr spectroscopy is well suited to the study of cyclodextrin radical association in solution; nitroxide radicals undergo complexation with cyclodextrins in aqueous solution, and inclusion is strongly dependent on the steric factor and functional groups polarity. Inclusion equilibrium thermodynamic data are easily reached when a biradical with large dipolar splitting is used.[49]

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