Nitroxides. 73. Electron Spin Resonance Study of Chiral Recognition by Cyclodextrin

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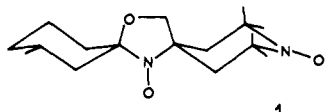
Abstract: The spectral changes associated with the complexation of biradical **1** (dispiro[(2,2,6,6-tetramethylpiperidine-1-oxyl)-4,4′-(oxazolidine-3′-oxyl)-2′,1″-(3″-methylcyclohexane)] with β-cyclodextrin have been used to show the difference of complexation of (+)- and (−)-fenchone by β-cyclodextrin: in water/dimethyl sulfoxide (1/1 by volume) and in the presence of β-cyclodextrin, biradical **1** shows a spectrum in which four peaks can be attributed to the biradical included in cyclodextrin. The intensity of these peaks decreases when fenchone is added, more with (+)-fenchone than with (−)-fenchone. This shows that (+)-fenchone is more complexed by β-cyclodextrin than (−)-fenchone. A precipitation experiment is in agreement with the spectroscopic result.

Electron spin resonance (ESR) is well suited to the study of the association with cyclodextrins in solution.[1,2] We have shown that the thermodynamic data of the association equilibrium can be determined when a biradical with a large dipolar splitting is used.[2]

It has been shown that cyclodextrins complex selectively one of the two enantiomers of a chiral (diamagnetic) molecule;[3-7] for example, (*R*)-(+)-2-methylcyclohexanone is selectively included in β-cyclodextrin.[6] In these cases the precipitating inclusion complex is enriched in one enantiomer. Recently[8] cyclodextrins have been used as chiral nuclear magnetic resonance shift reagents; CF_3 resonance occurs at higher field for (*S*)-(+) enantiomer than for (*R*)-(−)-1-phenyl-2,2,2-trifluoroethanol. This frequency shift does not give information on which enantiomer is more complexed; this should be derived from the peak heights. No direct spectroscopic evidence for chiral recognition in solution has been found with optically active nitroxide monoradicals.[1] In this article, we want to show chiral recognition in solution by cyclodextrin using ESR.

Since the ESR spectrum of a nitroxide biradical with large dipolar splitting *D* is easy to analyze,[9] we have used biradical **1**[10] (*D* = 225 Gauss in ethyl alcohol) to study the selective



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complexation by β-cyclodextrin of the two enantiomers of a chiral diamagnetic molecule ((+)- and (−)-fenchone) by the displacement method.[11,12]

Experimental Section

The ESR spectra were taken on a Varian V 4501 A ESR spectrometer.

Dispiro[(2,2,6,6-tetramethylpiperidine)-4,4′(oxazolidine)-2′,1″-(3″-methylcyclohexane)]. 3-Methylcyclohexanone (0.56 g) and 4-hydroxymethyl-4-amino-2,2,6,6-tetramethylpiperidine[13] (0.93 g) were boiled for 500 h in a benzene solution containing 10 mg of *p*-toluenesulfonic acid. Water was removed by azeotropic distillation. The mixture was extracted to give 0.80 g of viscous oil (yield 54%): NMR ($CDCl_3$) CH_3 (2,2,6,6) 1.1 and 1.33, CH_2 (3,5) 1.38 and 1.53, CH_2 (cyclohexyl) 1.8, CH_2 (oxazolidine) 3.53, CH_3 (3″) 1 ppm, J_{CH_3-H} = 5 Hz.

Dispiro[(2,2,6,6-tetramethylpiperidine-1-oxyl)-4,4′-(oxazolidine-3′-oxyl)-2′,1″-(3″-methylcyclohexane)]. The crude diamine (0.632 g) in ether solution was oxidized by *m*-chloroperbenzoic acid (1.17 g) in ether solution. The radical concentration was followed by ESR (oxidation time 7 h). When the ESR signal was maximum, the solution was washed with 5% sodium bicarbonate solution and dried over sodium sulfate. By thin layer chromatography (alumina activity III, 80% pentane–20% ether), 0.35 g of yellow-red crystals was obtained (yield 50%): mp 85°C; UV (methanol) λ 420 nm (ε ~ 16); ESR (benzene, 1×10^{-3} M) a broad line, 30 G width.

Anal. Calcd for $C_{17}H_{30}N_2O_3$: C, 65.77; H, 9.74; O, 15.56; N, 9.02. Found: C, 65.85; H, 9.71; O, 15.61; N, 8.95.

Precipitation Experiment. β-Cyclodextrin (2.26 g, 1.99×10^{-2} M) in 80 mL of water and 20 mL of Me_2SO[14] was warmed until complete dissolution. After cooling, 0.158 g of (±)-fenchone (1.04×10^{-2} M) was added. A white precipitate was formed. After 3 h, it was filtered and the filtrate extracted to give 0.08 g of fenchone, $[\alpha]^{20}_D$ −3.8 ± 0.2°.

ESR Results. At 15 °C, the ESR spectrum of biradical **1** (2.5×10^{-4} M) in a water/dimethyl sulfoxide (1/1 by volume) solution in presence of (+)- or (−)-fenchone (0.5×10^{-2} M) shows three narrow lines attributed to traces of monoradicals (estimated to be less than 3%) and a broad line of ca. 50 G width analogous to a published spectrum.[9] Biradical rotational correlation time is 1.5×10^{-10} s.[9]

Figure 1 shows the ESR spectra obtained at 15 °C in the presence of cyclodextrin (10^{-2} M) in water/dimethyl sulfoxide (1/1 by volume) solution containing biradical **1** (2.5×10^{-4} M), β-cyclodextrin (10^{-2} M), and (a) (+)-fenchone (0.5×10^{-2} M) or (b) (−)-fenchone (0.5×10^{-2} M). In the central part of the spectrum, three narrow lines (C, D, E) separated by 15 G are attributed to traces of monoradicals; four other lines (A, B, F, G) (AG = 432, BF = 200 G) are attributed to the biradical associated with cyclodextrin (complexed biradical rotational correlation time is ca. 2×10^{-9} s[9]). The central part of the spectrum is also perturbed by the broad line due to the uncomplexed biradical.

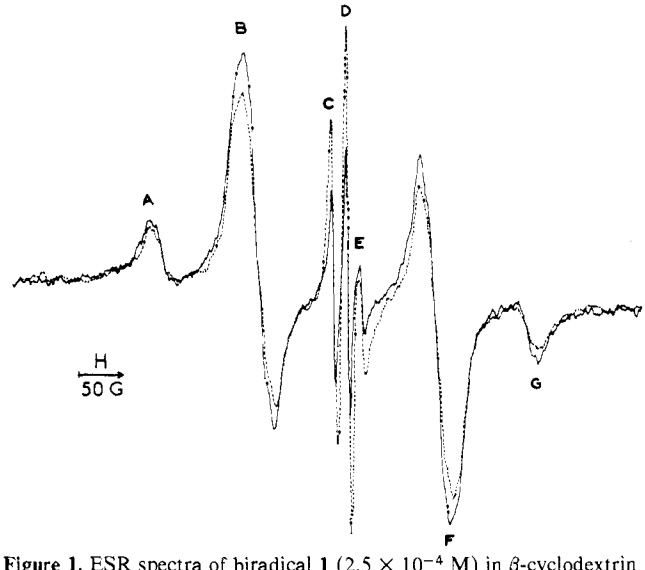

Figure 1. ESR spectra of biradical **1** (2.5×10^{-4} M) in β-cyclodextrin solution (10^{-2} M): . . . , in presence of (+)-fenchone (0.5×10^{-2} M); —, in presence of (−)-fenchone (0.5×10^{-2} M).

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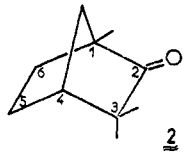
The peak heights of the A, B, F, and G lines are larger in the presence of (−)-fenchone than in the presence of (+)-fenchone (reproducibility in ten experiments is better than 5%). It can be concluded that (+)-(1*S*,4*R*)-fenchone (**2**) is preferentially included in β-cyclodextrin in



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50% water–50% Me_2SO at 15 °C. We have checked this result by a precipitation method; when racemic fenchone is complexed with β-cyclodextrin, the filtrate is enriched in (−)-fenchone. If the concentration of the inclusion complex in solution is neglected, the optical rotatory power of the filtrate gives the optical purity P of the uncomplexed fenchone: $P = 0.054$.

The (+)-fenchone thus is selectively included in the precipitate, in agreement with ESR results. The precipitation experiment gives $K_d/K_1 = 1.3 \pm 0.2$ for fenchone–cyclodextrin association constants at 20 °C in solution containing 80% water–20% Me_2SO.

Conclusion

This is the first direct spectroscopic evidence of a difference in the association of a cyclodextrin with the two enantiomers of a chiral molecule: the specific association of a chiral diamagnetic molecule can thus be observed by studying the displacement of the association equilibrium of a nitroxide biradical with cyclodextrin. This suggest that ESR can similarly be used to study chiral recognition by other receptors.[15,16]

References and Notes

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(15) For a general review see J. F. Collins, *Annu. Rep. Prog. Chem. Sect. B*, **73**, 416 (1976); P. S. Portoghese, *Acc. Chem. Res.*, **11**, 21 (1978).
(16) We have recently measured the different equilibrium constant for the association of both enantiomers of an optically active biradical by ESR.

Avidin–Biotin Interaction. Synthesis, Oxidation, and Spectroscopic Properties of Linked Models

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Abstract: In order to help uncover the basis of the strong biotin–avidin binding and to identify any contributory biotin–tryptophan interaction, we have synthesized model compounds, Biot-C_3-Ind (**4a**) and Biot-C_4-Ind (**4b**), having trimethylene and tetramethylene chains between the biotin ring system and indole, by starting with (+)-biotin and (+)-homobiotin. The UV spectroscopic effect of the proximate biotin moiety in Biot-C_3-Ind compared with 3-propylindole, both in cyclohexane (with 1% ethanol), is similar to the effect of biotin bound to avidin. Interaction of the biotin ring system and indole was also evidenced by a red shift in λ_{em} in comparing the fluorescence emission of 3-propylindole with that of Biot-C_3-Ind or Biot-C_4-Ind in cyclohexane (1 or 0.1% ethanol), but the proximate biotin ring system caused no quenching of fluorescence. We have found no evidence of strong interaction between the biotin ring system and the indole of tryptophan such as would help account for the magnitude of the avidin–biotin association constant. The diastereomeric sulfoxides of Biot-C_3-Ind were prepared, and the stereochemistry of each was established. ^1H NMR spectral comparisons with the methyl esters of (+)-biotin and its two sulfoxides confirmed the stereochemical assignments, as did X-ray single-crystal analysis (the sequel). With the ^1H NMR chemical shift assignments and the X-ray-confirmed stereochemistry, we have developed correlations with ^{13}C NMR chemical shifts that should prove useful in assigning stereochemistry to other asymmetric sulfoxides. A quantitative study of the *N*-bromosuccinimide oxidation of model compounds and of avidin, biotin, and the avidin–biotin complex established the relative rates of oxidation and the stereochemistry of oxidation of the biotin moiety in various environments. The protection of the tryptophans in the avidin–biotin complex against NBS oxidation is due only partially to consumption of the oxidizing agent by the bound biotin. Biotin in the avidin–biotin complex is oxidized by aqueous NBS, yet it is more protected than free biotin in aqueous solution. Oxidation of biotin in the avidin–biotin complex yields predominantly the α-sulfoxide, indicating steric limitation of the approach of oxidant to bound biotin.

The avidin–biotin complex,[1] which is one of the tightest biological complexes known, is characterized by a dissociation constant, K_D, of approximately 10^{-15} M.[2] The high affinity of avidin for biotin explains the toxic effect of ingested uncooked egg white in animals,[3] since it renders biotin (vitamin H), an essential growth factor,[4,5] unavailable to the test animals. Avidin binds not only biotin and its derivatives and analogues[1] but also biotin conjugated to protein, which makes avidin a very useful tool for the study of biotin-dependent enzymes.[6-8] Biotin- or biocytin-bound solid supports serve as effective affinity chromatographic columns for the purification of avidin,[9,10] and avidin–Sepharose columns can be used for the purification of biotinyl enzymes[11-13] or for the separation of subunits of biotinyl enzymes.[14] A general application of avidin–biotin complexation has been described by Hofmann in which biotin is used as an anchor to bind biological ligands

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