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(16) Large ($>10^3$) association constants as measured by the extraction technique were observed between any picrate and the complex 9, suggesting that the picrate ion itself is involved in bonding.

Nitroxides. 87. ESR Determination of the Thermodynamic Data for the Association of Two Paramagnetic Enantiomers with β-Cyclodextrin

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Abstract: Starting from (*R*)-(+)-3-methylcyclohexanone (and from racemic 3-methylcyclohexanone) (1″*R*,3″*R*)- (and racemic) dispiro[2,2,6,6-tetramethylpiperidine-1-oxyl]-4,4′-(oxazolidine-3′-oxyl)-2′,1″(3″-methylcyclohexane) have been prepared. Their complexation with β-cyclodextrin has been studied by electron spin resonance and the association constants of the two enantiomers have been determined, thus providing direct spectroscopic evidence for the enantiomeric selectivity in the complexation by cyclodextrin. The ratio of association constants measured by ESR is similar to the ratio of association constants of related diamagnetic enantiomers of the 3-methylcyclohexanone.

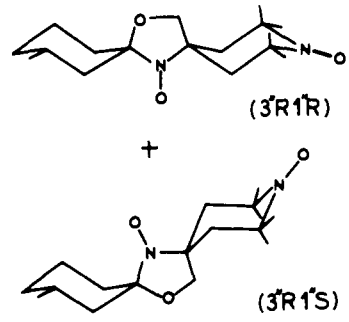
In solution, cyclodextrins form inclusion complexes without covalent binding with many molecules.[1-5] They selectively complex one of the two enantiomers of an optically active molecule; the precipitating inclusion complex is enriched in one of the enantiomers.[6a,b]

Cooper and Mac Nicol have shown by microcalorimetry a distinct discrimination in the binding of optical isomers.[7] Recently, we have used an ESR displacement method to show this selective association in solution with diamagnetic enantiomers.[8]

In this article, we want to determine by ESR the association constants K^+ and K^- of β-cyclodextrin with the two enantiomers of an optically active *paramagnetic* molecule. Since biradicals with large dipolar splitting allow an easy determination of the inclusion equilibrium thermodynamic data,[5] we have chosen to study the inclusion of an optically active nitroxide biradical in β-cyclodextrin.

This biradical (B) has been prepared from 3-methylcyclohexanone and a biradical spin label.[8-10] Two forms have been obtained: an optically active form (B*d*) from (*R*)-(+)-3-methylcyclohexanone and a racemic form (B*dl*) from racemic (±)-3-methylcyclohexanone. In principle two epimers can be obtained (Scheme I) in which the nitrogen is cis or trans to the methyl substituent in the cyclohexane ring [starting from (*R*)-(+)-3-methylcyclohexanone, these two epimers are 1″*R*,3″*R* and 1″*S*,3″*R*]. In each case, a single product has been obtained and shown to be unique by chromatography and recrystallization. The two forms have the same nuclear magnetic resonance spectra.[8] By comparison with the NMR spectra of oxazolidinic monoradicals,[11-13] it can be deduced that the cyclohexane ring is in a chair conformation and that the nitrogen and the methyl group are both in equatorial position. This shows that the single product obtained is the 3″*R*,1″*R* biradical from the 3*R* ketone and the racemic mixture of 3″*R*,1″*R* and 3″*S*,1″*S* biradicals from the racemic ketone.

Scheme I



Electron Spin Resonance Study

The ESR spectra have been recorded on a Varian E 12 spectrometer equipped with a variable-temperature accessory. Samples have been prepared by adding 10 μL of a solution of biradical B*d* (or B*dl*) in dimethyl sulfoxide (Me_2SO) to 1 mL of a Me_2SO/water (1/1 by volume) mixture or to 1 mL of a β-cyclodextrin solution in the same solvent. The following concentrations have been used: (I) β-cyclodextrin 5×10^{-2} M, B*d* (or B*dl*) 10^{-3} M; (II) β-cyclodextrin 10^{-2} M, B*d* (or B*dl*) 0.25×10^{-3} M.

In the absence of cyclodextrin, biradical B*d* (or B*dl*) (10^{-3} M in the Me_2SO/water solvent) shows a single broad line of ca. 40 G width (from which a rotational correlation time can be estimated, $\tau_c \simeq 10^{-10}$ s).[14] In the presence of cyclodextrin (5×10^{-2} M) at 20 °C, biradical B*d* (10^{-3} M) shows the spectrum presented in Figure 1. At the center of the spectrum, three narrow lines (c, d, e) superimposed on a broad line can be observed: we assign these three narrow lines to monoradical traces (less than 3% of biradical) and the broad line to uncomplexed biradical. On each side of the central lines, four lines (a, b, f, g) are observed, the symmetrical lines being separated

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Table I. Association Constants Kd, Kdl, and Kl of Biradical Bd, Bdl, and Bl with β-Cyclodextrin: (1) β-Cyclodextrin (5×10^{-2} M) and Bd or Bdl (10^{-3} M); (2) β-Cyclodextrin (10^{-2} M) and Bd or Bdl (0.25×10^{-3} M)[a]

	(1)				(2)			
T, K	$K(d)$	$K(dl)$	$K(l)$	$\rho = K(d)/K(l)$	$K(d)$	$K(dl)$	$K(l)$	$\rho = K(d)/K(l)$
273	81	42	24	3.4	78	39	21	3.7
278	62	35	21	3	63	33	18	3.5
283	45	27	17	2.6	49	27	15	3.3
288	32	23	16	2	40	24	14	2.9
293	24	17	12	2	27	18	12	2.3
298	21	13	9	2.3	23	15	11	2.1
303	15	12	10	1.5	18	12	9	2

[a] The uncertainty in K is estimated to be 20%.

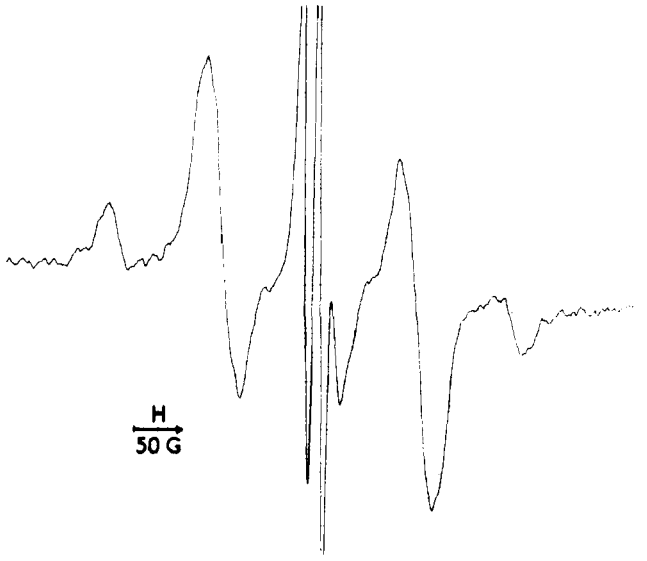

Figure 1. ESR spectrum of biradical Bd (10^{-3} M) in presence of β-cyclodextrin (5×10^{-2} M) at 20 °C.

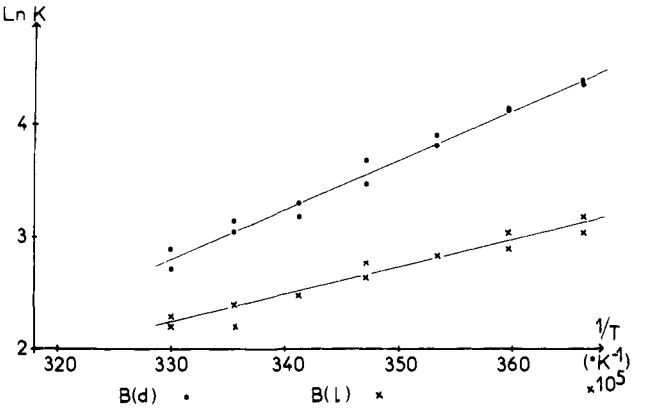

Figure 2. Variation of log K as a function of $1/T$ for biradical Bd (•) and Bl (×) in β-cyclodextrin solution.

by 200 and 432 G. These four lines are attributed to the complex biradical ($\tau_c \simeq 2 \times 10^{-9}$ s).[14]

If A, B, and C are the concentration of associated biradical, uncomplexed biradical, and cyclodextrin, the association equilibrium constant is $K = A/BC$.

If B_0 and C_0 are the initial biradical and cyclodextrin concentration ($B_0C_0 \gg A^2$)

$$K = A/(B_0 - A)(C_0 - A) = A/B_0C_0 - A(C_0 + B_0)$$

The association constant can be determined from the concentration A of included biradical. In the range of temperatures used, we have verified that the widths of ESR lines of included biradical do not change. The heights h_i of the i = a, b, f, g lines are then proportional to the concentration A of associated biradical. Since the b line is best resolved, A has been measured from this line: $A = \theta h$.

The proportionality constant θ has been determined by using different concentrations at the same temperature. The association constants K_d (and K_{dl}) of biradical Bd (and Bdl) with β-cyclodextrin have been calculated and are given in Table I. If Bl is the enantiomer of Bd, the respective association constants are K_d and K_l. From the same measures, the association constants K_d and K_l can be determined. In the experiment with the racemic biradical, initial concentrations in Bd and Bl are $B_0/2$. They become at equilibrium $B_0/2 - A\gamma$ and $B_0/2 - A(1 - \gamma)$, γ being the fraction of cyclodextrin combined with biradical Bd. The association constants are

$$K_d = \frac{A\gamma}{(B_0/2 - A\gamma)(C_0 - A)}$$

$$K = \frac{A(1 - \gamma)}{[B_0/2 - A(1 - \gamma)](C_0 - A)}$$

If k_d is determined by an experiment using Bd, the measure of A in the experiment using Bdl gives γ and K_l.

Table I gives K_d and K_l as a function of temperature. The numerical errors on K are estimated to be 20%. An Arrhenius plot (Figure 2) gives association enthalpy and entropy of d and l biradicals with cyclodextrin between 0 and 30 °C: $\Delta H_{assoc(d)} = -8.5 \pm 1.5$ kcal/mol; $\Delta S_{assoc}(d) = -23 \pm 3$ cal K^{-1} mol^{1}; $\Delta H_{assoc}(l) = -5 \pm 1$ kcal/mol; $\Delta S_{assoc}(l) = -11 \pm 3$ cal K^{-1} mol^{-1}.

These values are probably related to a better fit of the d enantiomer in the cyclodextrin cavity.

It may be interesting to compare the association constants ratio $\rho = K_d/K_l$ of biradical with β-cyclodextrin with the same ratio ρ' for 3-methylcyclohexanone. Since in analogous monoradicals we have shown that the cyclohexane ring is included in the cyclodextrin,[5] one may expect that chiral recognition comes mainly from the 3-methylcyclohexane residue. In such a case the values of ρ and ρ' should be approximately the same. We have determined ρ' by selective precipitation. By addition of a racemic compound to a solution of cyclodextrin, a precipitate is formed and filtered. The filtrate is extracted and its optical rotatory power $[\alpha']$ measured. If the concentration of the inclusion complex in the filtrate is neglected, $[\alpha']$ gives the optical purity P of the uncomplexed compound. Using the same notations as before (B_0' is now the initial concentration in racemic compound), the optical purity is

$$P = A(2\gamma - 1)/B_0' - A = [\alpha']/[\alpha]$$

then

$$\gamma = \tfrac{1}{2}[1 + P(B_0'/A - 1)]$$

$$\rho' = K_d/K_l = \frac{[B_0'/2 - (1 - \gamma)A]\gamma}{[B_0'/2 - \gamma A](1 - \gamma)}$$

In our experiment, starting from 550 mg of racemic ketone, 250 mg was extracted from the filtrate and showed $[\alpha']_D$

$-1.8°$ in ethanol,[15] thus leaving 300 mg in the cyclodextrin. Since we measured for optically pure (*R*)-(+)-3-methylcyclohexanone $[\alpha]_D$ +13.4°, in the same solvent[16]

$$\gamma = \tfrac{1}{2}[1 + 1.8/13.4(550/300 - 1)] = 0.56$$

and $\rho' = 1.7 \pm 0.25$ at 20 °C. This result can be compared to the value determined by ESR for the related biradical.

Conclusion

Evidence has been given by ESR for the selective inclusion in β-cyclodextrin of one of the two enantiomers of an optically active paramagnetic molecule. The two association constants have been determined. For paramagnetic molecules this method is more rapid and requires a smaller amount of material than the same determination by precipitation. The selectivity in the complexation is the same order of magnitude for racemic 3-methylcyclohexanone and the cognate racemic biradical. This is consistent with a chiral recognition based on the 3-methylcyclohexane residue in both compounds.

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Role of Buffers in a Methylase Model Reaction. General Base Catalysis by Oxyanions vs. Nucleophilic Dealkylation by Amines

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Abstract: The *cis*-cyclopentanol derivative, **1**, was synthesized as a model for the *O*-methylation of ribose of tRNA, as catalyzed by tRNA 2′-*O*-methyltransferase. The decomposition of **1** in oxyanion buffers was studied over a wide pH range, at 25 and 40 °C. This reaction exhibits a plateau rate at low pH (k_{ROH}) and a hydroxide dependence at higher pH, associated with k_{RO^-}. Thermodynamic data gathered from a study of k_{ROH} and k_{RO^-} at five temperatures (25–40 °C) gave $\Delta S^{\ddagger} = -15$ eu for the k_{ROH} reaction and −3.7 eu for the k_{RO^-} reaction. Kinetic studies and product analysis data for the reaction of **1** lead to the conclusion that **1** cyclizes in an intramolecular fashion to give *cis*-2-oxabicyclo[3.3.0]octane (**3**) and *p*-nitrothioanisole (**12**) in a reaction which is catalyzed by the added buffer base. The fact that the trans analogue, **2**, which cannot undergo intramolecular alkylation, is inert in oxyanion buffers rules out any participation by buffer base in an intermolecular reaction. When the reaction of **1** is carried out in amine buffers, however, both *p*-nitrothioanisole and (2-*cis*-hydroxycyclopentyl)ethyl *p*-nitrophenyl sulfide (**4**) are detected in the reaction mixture by high-pressure liquid chromatographic (LC) analysis. The amount of each compound formed is dependent upon the amine used, the total buffer concentration, and the ratio of basic to acidic buffer species. When **2** reacts in amine buffers, *p*-nitrothioanisole and (2-*trans*-hydroxycyclopentyl) ethyl *p*-nitrophenyl sulfide (**5**) are formed, but the product ratios (**5**:**12** = ca. 9:1) are not dependent on buffer concentration, as with the reaction of **1**. Such product analysis data, as well as a comparison of the rates of reaction of **1** with those of other sulfonium compounds, indicate that amine buffers preferentially effect nucleophilic demethylation rather than function as general base catalysts, as do the oxyanions. A β value of 0.27 is obtained for all oxyanions and amines studied, with the exception of imidazole. No nucleophilic catalysis by imidazole of the demethylation of **1**, **2**, or dimethyl-*p*-nitrophenylsulfonium perchlorate (**6a**) could be demonstrated.

Introduction

The transfer of an intact methyl group from a suitable donor to a suitable acceptor is a vitally important biological process. With the exception of the role played by 5-methyltetrahydrofolic acid in methionine biosynthesis,[1] the universal donor of intact methyl groups is *S*-adenosylmethionine (AdoMet).[2] AdoMet is an important cofactor for enzymes that methylate a diverse array of nucleophilic acceptors within the cell (i.e., DNA, RNA, proteins, biogenic amines, etc.)[3] and has been implicated, by the fact that it may promote elevated levels of potentially psychotogenic methylated amines, as a potential causative agent in schizophrenia.[4]

Following a series of kinetic studies on catechol *O*-methyltransferase (COMT, EC 2.1.1.6), Coward and co-workers postulated that one of the two hydroxyl groups of a suitable catechol substrate could function as an intramolecular general base catalyst to facilitate methylation by AdoMet of the other hydroxyl group.[5] The proposed mechanism is shown below for this methylation. Also shown below is a possible mechanism involving intramolecular general base catalysis in the meth-

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