

crosslinking to prevent unfolding.

In terms of the potential application of the urokinase conjugate as a therapeutic agent, we examined the inhibitory effect of blood plasma on the fibrinolytic activity of the conjugate and *in vitro* biological stability using human plasma. We found that *in vitro* biological half-life increased about 40 times longer. Also considering the conjugate as a potential fibrinolytic agent, the resistance to plasma inhibition is important.^{18,19} At the concentration of 5 μ l of plasma in the reaction mixture which corresponds to the concentration of plasma in blood, 30% activity of the conjugate was inhibited, while 90% activity of the native urokinase was inhibited.

In conclusion, urokinase-dextran conjugate showed high fibrinolytic activity, good stability on pH, temperature and storage, and excellent *in vitro* biological half-life with human plasma. Specially, it can provide injectable form. Therefore, it shows good potential as a fibrinolytic agent as well as a blood volume expander.

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Platinum(II) Complexes of 2,2'-Diaminobinaphthyl

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Platinum(II) complexes of R-2,2'-diaminobinaphthyl (R-dabn), [Pt(R-dabn)(H₂O)₂]Cl₂, [Pt(R-dabn)(R-Pn)]Cl₂, [Pt(R-dabn)(R-bn)]Cl₂, and platinum(II) complexes of S-2,2'-diaminobinaphthyl (S-dabn), [Pt(S-dabn)(H₂O)₂]Cl₂, [Pt(S-dabn)(S-Pn)]Cl₂, and [Pt(S-dabn)(S-bn)]Cl₂ have been prepared. (R-Pn and S-Pn are, respectively R- and S isomer of 2,3-diaminobutane). R-Pn and S-bn are, respectively R and S isomer of 2,3-diaminopropane). In the vicinity of the B-absorption band region of dabn, the circular dichroism spectra of platinum(II) complexes of R-dabn series show a positive B-band followed by a negative higher energy A-band, which is generally understood as the splitting pattern for a λ conformation, while the circular dichroism spectra of platinum(II) complexes of S-dabn series show a negative B-band followed by a positive higher energy A-band in the long-axis polarized absorption region as expected for a d conformation.

Introduction

The various methods have been applied to the determination of absolute configurations of metal complexes. Among the methods being used frequently are as following^{1,2};

(a) Empirical and semiempirical regional rules including octant sign method, ring-pairing method, and sextent rule,

(b) Chiroptical spectroscopic methods

(i) Empirical methods based on the d-d transition utilizing circular dichroism (CD) and optical rotatory dispersion (ORD) spectroscopic data

(ii) Nonempirical method based on exciton theory (exciton splitting theory),

- (c) X-ray crystallographic method,
- (d) Nuclear magnetic resonance
- (e) Miscellaneous techniques such as chemical correlation and active racemate method.

The nonempirical determination of absolute configuration has been applied to the metal complexes of particular ligands containing two noncoplanar chromophores,³⁻¹² and has been successful in predicting the absolute configuration of coordination compounds with conjugated ligands such as 1,10-orthophenanthroline (phen)^{4,5,13}, 2,2'-bipyridyl (bipy)^{6-8,13} and bianthryl¹² whose electric dipole $\pi-\pi^*$ transitions lie in the near ultraviolet region. This nonempirical method has so far been utilized to determine the absolute configuration of octahedral metal complexes only³. In the present work a nonempirical method based on exciton theory is proposed in a qualitative fashion for determination of absolute configuration of metal complexes in square planar symmetry. 2,2'-diaminobinaphthyl(dabn) which contains a skewed biaryl group, is chosen in this work as the ligand having non-coplanar chromophores.

In the free ligand the electric dipole transitions are polarized in the molecular plane—along either the short axis (X-polarized) or the long axis (Y-polarized) as shown in Figure 1. When two or three such ligands coordinate to form a *cis-bis* or a tris complex, the long axis polarized transitions can couple to yield two transitions which have both electric and magnetic dipole moments making them optically active transitions. The absorption spectrum retains the character of the ligands with corresponding increase in intensity to account for the coordination of two or three ligands. One of the resultant transitions possesses A_2 symmetry and is directed along the C_3 axis of the tris-complex; the other possesses E symmetry perpendicular to the C_3 axis. The splitting between the A_2 excited state and the E excited state is termed exciton splitting.^{3,4,6,7}

In order to apply the nonempirical method it is essential that under a given ligand absorption band the exciton splitting produce two CD bands of about equal areas and opposite signs. A positive CD will be produced by a right-hand helical displacement for the E transition, which is perpendicular to the C_3 axis, whereas a negative CD will result from a left-hand helical displacement for the E component. All that remains is the correct assignment of the E and A_2 components of a tris complex.

The nonempirical assignment of absolute configuration to tris-phenanthroline and tris-bipyridyl complexes of Ru(II), Ru(III) and Fe(II) has been proved correct when the X-ray structural determination of $[\text{Fe}(\text{bipy})_3]^{2+}$ was made and a chemical correlation¹⁴ of $(+)-[\text{Ru}(\text{phen})_3]^{2+}$ with $(+)-[\text{Ru}(\text{bipy})_3]^{2+}$ was investigated. Dollimore and Gillard⁹ resolved $[\text{Co}(\text{en})(\text{phen})_2]^{3+}$ recently. They found that the absolute configuration assigned on the basis of exciton splitting was the same as that predicted from the long wavelength component of the CD spectrum.

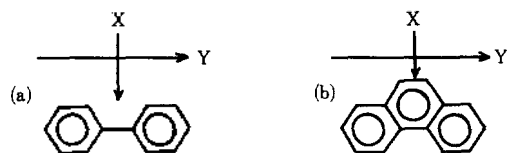


Figure 1. Long- and short-axis polarization for (a) 1,10-orthophenanthroline and (b) 2,2'-bipyridyl.

Results and Discussion

When the noncoplanar electric dipoles located in each half of the biaryl couple, a magnetic transition moment will be generated. Electrostatic coupling of these electric transition dipole moments results in a helical displacement of charge. The helicity depends on the symmetry of the transition. Consequently, two circular dichroism bands are found with opposite signs and almost equal areas under the corresponding absorption band.

Figure 2 shows the λ -configuration and the coordinate axes of 2,2'-diaminobinaphthyl. The x and y axis are in the plane of the paper. The z axis is perpendicular to the plane of the paper. The dotted portion is below the plane and the dihedral angle is less than 90° .

The electronic states of binaphthyl are either symmetric, A, or antisymmetric, B, with respect to rotation by π about the z-axis. A transition from the ground state of 2,2'-diaminobinaphthyl to an excited state with A-symmetry will have its electric dipole moment directed along the z-axis, if the transition is long-axis polarized. Long-axis transitions of B-symmetry have moments perpendicular to the z-axis. Figure 3 describes the orientation of the electric dipole moments for long- and short-axis polarization. Coupled transitions with A-symmetry are shown with solid arrows, and those with B-symmetry dotted arrows.

The electric dipole moment can be described by two orthogonal components. When the binaphthyl takes the λ -configuration, the long-axis transition with A-symmetry will have one component on each of the two naphthalene halves, directed along the z-axis. The other components will be antiparallel and produce a counterclockwise displacement of charge, with a magnetic moment along z. Thus the A-transition should be seen as a negative CD band. For the transition with B symmetry the parallel components will be along the x-axis, whereas the antiparallel components will produce a clockwise displacement of charge along x. A positive CD band will result from the B-transition. Grinter and Mason calculated the A band

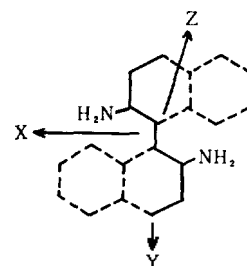


Figure 2. Coordinate axes for 2,2'-diaminobinaphthyl.

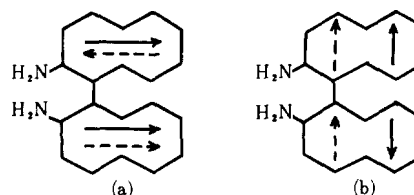


Figure 3. Orientation of dipole moments of 2,2'-diaminobinaphthyl excitations with (a) long-axis and (b) short-axis polarization. Solid arrows represent transitions with A-symmetry and hatched arrows are for the those with B-symmetry.

to be at a higher energy than the B band in the bianthrils.

Naphthalene reveals three bands at 34.2 kK (long $\epsilon = 3.64$), 35.6 kK (log $\epsilon = 3.82$), 36.8 kK (log $\epsilon = 3.89$) which have been assigned the p-band, short-axis polarization. A band at 40.8 kK (log $\epsilon = 5.02$) corresponds to the β -band of long-axis polarization. Examination of the absorption spectrum of $[\text{Pt}(\text{R-dabn})(\text{H}_2\text{O})_2]^{2+}$ in Figure 4 shows a slight shoulder centered at about 41.2 kK. This suggests the splitting of the β -band. The circular dichroism spectrum shown in Figure 4, indicates a positive CD band at 41.3 kK and a negative band at 42.8 kK. The negative band is not quite as intense as the positive band, but it lies on the high energy side of an intense positive band which may cause the loss of some negative rotational strength. According to Grinter and Mason's work on the closely related bianthrils a positive B-band followed by a negative higher energy A-band in the region of the long-axis polarized absorption band indicates a λ conformation. The R-dabn is known to have the λ configuration from the X-ray diffraction determination of the absolute configuration of (+)-2,2'-dihydroxy-1,1'-binaphthyl-3,3'-dicarboxylic acid dimethyl ester¹⁴ and subsequent chemical correlations of this compound to 2,2'-diaminobinaphthyl by Mislow et al.¹⁶ Figure 4 shows also the mirror image CD for $[\text{Pt}(\text{S-dabn})(\text{H}_2\text{O})_2]\text{Cl}_2$. The low energy B-band at 41.3 kK is negative and the high energy A-band at 42.8 kK is positive as expected for the δ conformation.

The CD spectra of $[\text{Pt}(\text{R-dabn})(\text{R-Pn})]\text{Cl}_2$ and $[\text{Pt}(\text{S-dabn})(\text{S-Pn})]\text{Cl}_2$ are shown in Figure 5. An inspection of the CD spectrum of $[\text{Pt}(\text{R-dabn})(\text{R-Pn})]\text{Cl}_2$ reveals a high energy negative band at 45.4 kK and a positive band at 42.6 kK. The CD spectrum of $[\text{Pt}(\text{R-dabn})(\text{R-bn})]\text{Cl}_2$ shown in Figure 6 closely resemble that of $[\text{Pt}(\text{R-dabn})(\text{R-Pn})]\text{Cl}_2$. The splitting pattern corresponds to the λ dabn ligand conformation. The mirror image CD spectra of $[\text{Pt}(\text{S-dabn})(\text{S-Pn})]\text{Cl}_2$ and $[\text{Pt}(\text{S-dabn})(\text{S-bn})]\text{Cl}_2$ show a low energy negative B-band at 42.6 kK follow-

ed by a high energy positive A-band at 45.4 kK as predicted for the δ dabn ligand conformation.

It is noted from a molecular model of the free ligand that the R-dabn ligand can coordinate only in the λ conformation while the S-dabn only in the δ conformation. Such study using molecular models lends further support to the nonempirical determination of absolute configuration for skewed biaryls.

Experimental

2,2'-Diaminobinaphthyl(dabn) was prepared and resolved by known methods.^{17,18} 1,2'-Diaminopropane(pn) and 2,3'-diaminobutane(bn) were resolved by the method known in the literature.^{19,20}

$[\text{Pt}(\text{R-dabn})(\text{H}_2\text{O})_2]\text{Cl}_2 \cdot \text{H}_2\text{O}$. 0.04g of R-dabn was dissolved in 60 ml of warm ethanol, and resulting solution was added dropwise to a solution of 0.58g of K_2PtCl_4 in 50 ml of water. The reaction mixture was heated and stirred at a temperature

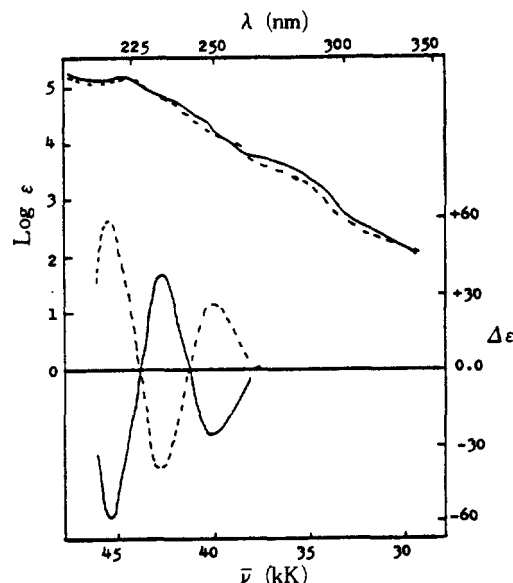


Figure 5. Electronic absorption and CD spectra of $[\text{Pt}(\text{R-dabn})(\text{R-Pn})]\text{Cl}_2$ (—) and $[\text{Pt}(\text{S-dabn})(\text{S-Pn})]\text{Cl}_2$ (---).

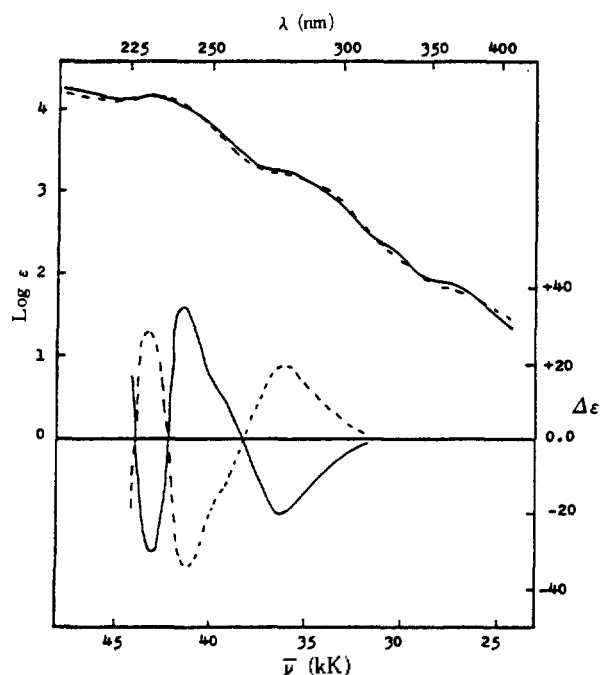


Figure 4. Electronic absorption and CD spectra of $[\text{Pt}(\text{R-dabn})(\text{H}_2\text{O})_2]\text{Cl}_2$ (—) and $[\text{Pt}(\text{S-dabn})(\text{H}_2\text{O})_2]\text{Cl}_2$ (---).

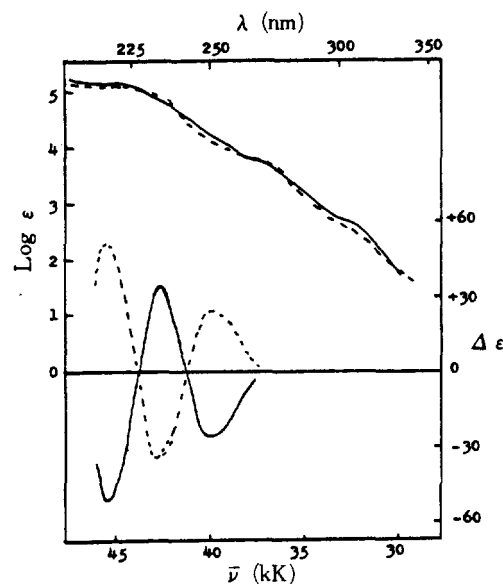


Figure 6. Electronic absorption and CD spectra of $[\text{Pt}(\text{R-dabn})(\text{R-bn})]\text{Cl}_2$ (—) and $[\text{Pt}(\text{S-dabn})(\text{S-bn})]\text{Cl}_2$ (---).

of 60-70°C for two hours. The mixture was cooled and HClO₄ (11.5N) was added dropwise to this solution until the pH of the solution became 2. The solution was allowed to stand at room temperature for 5 hours. The solution was concentrated on a rotary evaporator until crystallization. The mixture was cooled in an ice bath. The product was collected by filtration and recrystallized once from warm water. Yield 0.44g (52%). *Anal.* Calcd. for Pt(C₂₀H₁₆N₂) (H₂O)₂ Cl₂·H₂O : C, 39.74; H, 3.64; N, 4.64; Cl, 11.75 Found. C, 39.77; H, 3.60; N, 4.65; Cl, 11.69.

[Pt(S-dabn) (H₂O)₂]Cl₂·H₂O. This was prepared by the same method as that used for [Pt(R-dabn) (H₂O)₂]Cl₂·H₂O except that s-dabn was used in place of R-dabn.

[Pt(R-dabn) (R-pn)]Cl₂. 0.30g of [Pt(R-dabn) (H₂O)₂]Cl₂·H₂O and 0.04g of R-pn were suspended in 300 ml of water. The mixture was stirred and heated at a temperature of 70-75°C for 10 hours. Gradually the suspension dissolved. The solution was cooled and filtered to remove unreacted reactant. It was then concentrated on a rotary evaporator until crystals were formed. The mixture was placed in an ice bath. The white crystals were collected on a sintered glass filter and washed successively with ice-cold water, ethanol, ether, and dried in air. The product was recrystallized from warm water. Yield 0.14g (46%) *Anal.* Calcd for Pt(C₂₀H₁₆N₂) (C₃H₁₀N₂)Cl₂ : C, 44.23; H, 4.17; N, 8.97; Cl, 11.38. Found : C, 44.29; H, 4.18; N, 9.00; Cl, 11.35

[Pt(S-dabn) (S-pn)]Cl₂. This was prepared by the method described above using [Pt(S-dabn) (H₂O)₂]Cl₂·H₂O and S-pn:

[Pt (R-dabn) (R-bn)]Cl₂. Procedure was the same as that used for [Pt(R-dabn)-(R-pn)]Cl₂ except that the starting materials were [Pt(R-dabn) (H₂O)₂]Cl₂·H₂O and R-bn. *Anal.* Calcd. for Pt(C₂₀H₁₆N₂) (C₄H₁₂N₂)Cl₂ : C, 45.14; H, 4.39; N, 8.78; Cl, 11.13. Found : C, 45.18; H, 4.41; N, 8.80; Cl, 11.19.

[Pt(S-dabn) (S-bn)]Cl₂. Procedure was the same as for [Pt(R-dabn)-(R-pn)]Cl₂ except that the starting materials were [Pt(S-dabn) (H₂O)₂]Cl₂ and S-bn.

Instruments Used. The electronic absorption spectra were obtained using a Cary 17 UV-Visible Spectrophotometer. Circular dichroism spectra were measured using a Jasco Model 20 ORD/CD Spectrophotometer.

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