and eluted with 5×10^{-4} M HBF_4. The main orange band was collected, rotoevaporated to dryness, and analyzed (see Results).

Methods. HPLC was performed on a Waters Associates liquid chromatography system equipped with a variable-wavelength UV detector and a fixed-wavelength detector, in order to monitor two different wavelengths simultaneously when needed. A solvent system containing water/methanol with up to 0.2% TFA was used for the elution of cobalt complexes of hydrophobic peptides. The progress of each reaction was followed by using Radial Pak C_{18} cartridges (10-μm octadecylsilane columns, 8 mm × 10 cm, Waters) and a Waters Radial Compression (Model RCM 100) or Altex C_{18} columns (5 μm, 4.6 mm × 25 cm) with flow rates of 1-2 mL/min.

Differential pulse measurements were done on the complexes by using a PAR Model 174A polarographic analyzer, and cyclovoltammograms were recorded with a PAR Model 173 potentiostat, a Model 175 universal programmer, and an Omnigraph 2000 X-Y recorder. Detailed electrochemical measurements were done by using differential pulse polarography because of the presence of two overlapping waves. A Pt-button working electrode was used in a three-electrode configuration with SCE as the reference electrode.

Kinetic Experiments. Solutions of the binuclear complexes of 0.5×10^{-3} to 1.5×10^{-3} M were freshly prepared by dissolving the appropriate weight of the complex in 1.8 mL of 1 M HTFA and degassing the resulting solution with argon in a Zwickel flask for 30 min. In a separate flask $[(NH_3)_6Ru]Cl_3$ was dissolved in 0.1 M HTFA and reduced over Zn/Hg, and 0.2 mL of this solution was added to the binuclear complex to generate the desired concentration of the Ru^{II}-L-Co^{III} precursor complex ($\sim 5 \times 10^{-5}$ M). The rate of intramolecular electron transfer was monitored spectrophotometrically at $\lambda = 480$ nm for all the reactions studied. The temperature was controlled by using a thermostated cell compartment. For the fast reactions ($k > 10^{-4}$ s^{-1}), data were collected for the entire reaction. For the slow reactions, data were collected for up to 10% reaction. The reactions were then quenched with a slight excess of ammonium persulfate and the absorbance (A_∞) was measured immediately. The ratio of (Ru^{III}-L-Co^{III})/(Ru^{II}-L-Co^{III}) was always maintained between 10 and 25. This was found to be necessary to prevent interference of the product formed during the reaction.[15]

The intermolecular reactions were also studied in an argon atmosphere. The $[OH_2(NH_3)_4Ru^{II}$-isn) solution (where isn = isonicotinamide), prepared by reducing the corresponding $[SO_4(NH_3)_4Ru^{III}$-isn] with $[(NH_3)_6Ru^{II}]$,[15] was added to the $[(NH_3)_5Co(GlyGly)](BF_4)_3$ to make the final concentration of the ruthenium complex 5×10^{-5} M and the final concentration of $[(NH_3)_5Co(GlyGly)]^{3+}$ 5×10^{-3} M in 1 M HTFA. The decrease in absorbance was monitored at $\lambda = 480$ nm. Pseudo-first-order rate constants k_{obsd} (s^{-1}) and second-order rate constants k_b (M^{-1} s^{-1}) were evaluated from the absorbance vs. time data.

Treatment of Data. Rate constants for the fast reactions ($k > 10^{-4}$ s^{-1}) were obtained from the slopes of the least-squares plots of ln ($A_\infty - A_t$) vs. t. For the slow reactions, the initial rate method was used. $\Delta H^\ddagger$ was calculated from the least-squares fit of ln k/T vs. $1/T$ plots. The $\Delta S^\ddagger$ values were then calculated from the high and low limits of $\Delta H^\ddagger$.

Acknowledgment. This work was supported by the National Institutes of Health (Grant 26324). S.I. is the recipient of a National Institutes of Health Career Development Award (AM 00734) (1980-1985) and a Camille and Henry Dreyfus Teacher-Scholar Award (1982-1986). S.I. gratefully acknowledges helpful discussions with Professor H. Taube.

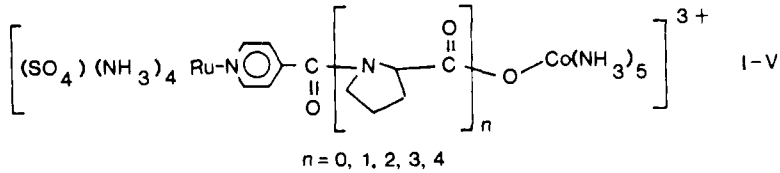
Registry No. $[(NH_3)_5Co(Gly\text{-}iso)Ru(NH_3)_4SO_4](BF_4)_3 \cdot HBF_4$, 88510-33-2; $[(NH_3)_5Co(Pro\text{-}iso)Ru(NH_3)_4SO_4](BF_4)_3 \cdot HBF_4$, 88510-36-5; $[(NH_3)_5Co(Phe\text{-}iso)Ru(NH_3)_4SO_4](BF_4)_3 \cdot HBF_4$, 88510-39-8; $[(NH_3)_5Co(GlyGly\text{-}iso)Ru(NH_3)_4SO_4](BF_4)_3 \cdot HBF_4$, 88496-22-4; $[(NH_3)_5Co(PhePhe\text{-}iso)Ru(NH_3)_4SO_4](BF_4)_3 \cdot HBF_4$, 88496-25-7; $[(NH_3)_5Co(GlyPhe\text{-}iso)Ru(NH_3)_4SO_4](BF_4)_3 \cdot HBF_4$, 88496-28-0; $[(NH_3)_5Co(GlyLeu\text{-}iso)Ru(NH_3)_4SO_4](BF_4)_3 \cdot HBF_4$, 88510-42-3; $[(NH_3)_5Co\text{-}iso\text{-}Ru(NH_3)_4SO_4](BF_4)_3$, 88547-64-2; $[(NH_3)_5Co(GlyGly)](BF_4)_3$, 68582-25-2; $[(NH_3)_5Co(GlyPhe)](BF_4)_3$, 68582-27-4; $[(NH_3)_5Co\text{-}Gly](BF_4)_3$, 68582-21-8; $[(NH_3)_5Co\text{-}Pro](BF_4)_3$, 68582-23-0; $[(NH_3)_5Co\text{-}Phe](BF_4)_3$, 81688-40-6; $[(NH_3)_5Co\text{-}(ProPro)](BF_4)_3$, 68582-29-6; $[(NH_3)_5Co\text{-}(GlyLeu)](BF_4)_3$, 88496-31-5; $[(NH_3)_5Co\text{-}(PhePhe)](BF_4)_3$, 88496-34-8; $[(NH_3)_5Co(GlyGly\text{-}iso)]^{2+}$, 88496-35-9; $[(NH_3)_5Co(GlyPhe\text{-}iso)]^{2+}$, 88496-36-0; $[(NH_3)_5Co(Gly\text{-}iso)]^{2+}$, 69421-30-3; $[(NH_3)_5Co(Pro\text{-}iso)]^{2+}$, 88496-37-1; $[(NH_3)_5Co(Phe\text{-}iso)]^{2+}$, 88496-38-2; $[(NH_3)_5Co(ProPro\text{-}iso)]^{2+}$, 88496-39-3; $[(NH_3)_5Co(GlyLeu\text{-}iso)]^{2+}$, 88496-40-6; $[(NH_3)_5Co(PhePhe\text{-}iso)]^{2+}$, 88496-41-7; isonicotinic acid trifluoromethanesulfonate, 88496-42-8.

Electron Transfer across Polypeptides. 3. Oligoproline Bridging Ligands

Stephan S. Isied* and Asbed Vassilian

Contribution from the Department of Chemistry, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903. *Received June 30, 1983*

Abstract: A series of cobalt(III)-L-ruthenium(III) binuclear complexes (I-V), with a bridging oligoproline peptide derivatized



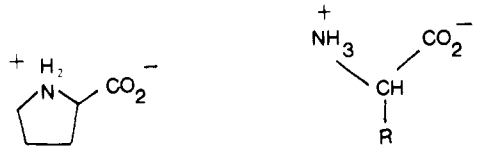
I-V

n = 0, 1, 2, 3, 4

with an isonicotinoyl (iso) group at the N-terminal, has been synthesized and purified by HPLC. These complexes provide a peptide spacer that separates the metal ions at distances determined by the peptide conformation and structure. Reduction of the above complexes to the precursor complex, the Co^{III}-L-Ru^{II} species, was followed by a slow rate of intramolecular electron transfer with unimolecular rate constants of 1.2×10^{-2}, 1.04×10^{-4}, 0.64×10^{-5}, 5.6×10^{-5}, and 1.4×10^{-4} s^{-1} for $n = 0$, 1, 2, 3, and 4. Over 2000 times variation in rate is seen for this series of binuclear complexes, which have identical reduction potentials, inner-sphere reorganization energies, and charge types. A decrease in rate by introducing $(Pro)_1$ and $(Pro)_2$ reflects an increase in the separation between the donor and acceptor. For the $(Pro)_3$ and $(Pro)_4$ compounds, the slow rate of electron transfer allows enough time for the conformation change of the proline to bring the two metal ions into close proximity, resulting in a more rapid rate of electron transfer.

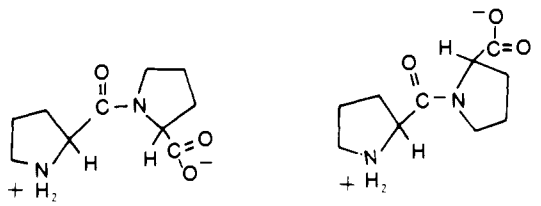
The amino acid proline (**1**) occupies a unique position among the other naturally occurring amino acids (**2**). The cyclic structure of its side chain restricts rotation about the C-N bond within a proline residue and also about the peptide bond formed between

0002-7863/84/1506-1732$01.50/0



1 2

the N terminal of a proline residue and any other amino acid. This results in a cis–trans conformational isomerism, with the proline being cis or trans to its next neighbor.

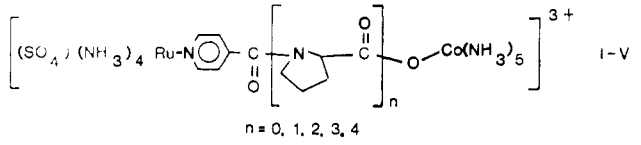


cis proline trans proline

This structural feature of proline results in a more rigid peptide backbone than that of any of the other naturally occurring amino acids. Solutions of polyproline polymers are known to exist in either the cis- or trans-helical structures, depending on solvent and ionic strength.[1,3] ^1H and ^{13}C NMR have been useful in assigning the structure of these cis–trans isomers.[1-4]

Energy-transfer studies across rigid molecules made use of oligoprolines as spacers for separating the donor and acceptor chromophores.[5,6] NMR and circular dichroism (CD) work showed that in aqueous acidic media proline oligomers (n = 2–5) are predominantly in the trans conformation.[1-3] Wuthrich et al.[3] have shown that as the acidity of aqueous solutions is increased, cis-proline peptides interconvert to the trans configuration, such that at pH 1 no cis prolines are detected. Strong interaction between the solvent (water) and the peptide carbonyl groups seems to stabilize the trans-helical structure.[1] Figure 1 shows the trans-helical structure of polyproline. In aqueous acidic solutions it is expected that the metal–ammine–oligoproline complexes I–V will also be predominantly in the extended trans configuration, with a fixed distance between the oxidant and reductant centers.[7]

In extending our studies on intramolecular electron-transfer rates across polypeptides,[8] we have used proline oligomers as a rigid class of peptides where the distance between the N and C terminals is more defined. This distinguishes this class of compounds from the flexible peptides of our previous study,[8] where the distance between the peptide terminals is harder to define. In order to measure the rate of intramolecular electron transfer across oligoprolines, we have synthesized and studied a series of metal complexes where the oligoproline units are bound to kinetically inert metal complexes of Co(III) and Ru(III) of the type



n = 0, 1, 2, 3, 4 I–V

In this paper we describe the synthesis, characterization, and

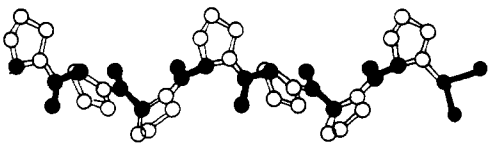


Figure 1. Trans-helical form of poly(L-proline). Dark circles represent the peptide bonds. Open circles in the 5-membered ring are the carbon atoms. (Adapted from ref 23.)

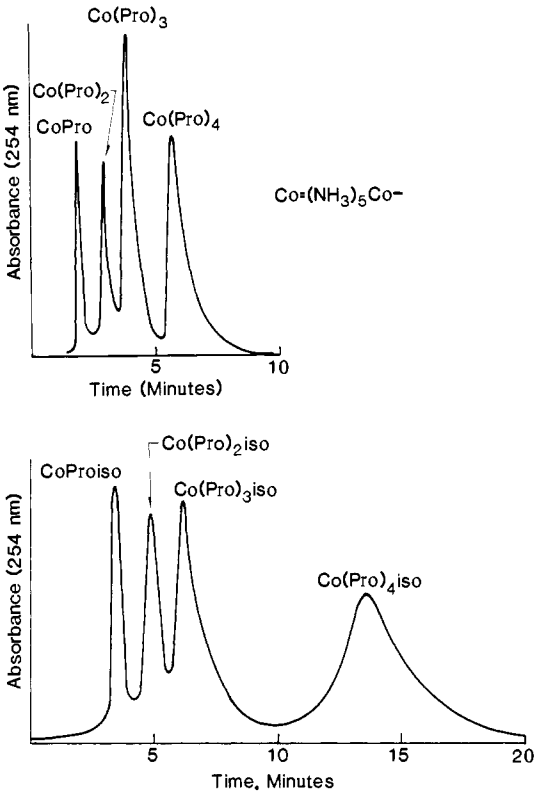


Figure 2. HPLC separation of $[(NH_3)_5Co(Pro)_n]^{3+}$ complexes (top) and the corresponding $[(NH_3)_5Co((Pro)_n\text{-iso})]^{3+}$ complexes (bottom). [Reverse phase RCM (waters) C-18 (10 μ), 40/60 MeOH/H_2O; 0.2% TFA, pH 2.54, adjusted with NaOH.]

Table I. Elemental Analyses for $[SO_4(NH_3)_4Ru\text{-(iso-(Pro)}_n\text{)-Co(NH}_3)_5](BF_4)_3\cdot HBF_4\cdot xH_2O$ (n = 1-4)[a]

compd	Pro bridge		% C	% H	% N	% Co	% Ru
II, x = 1	Pro	calcd	13.29	4.15	15.49	5.92	10.16
		obsd	12.91	4.02	14.41	6.00	10.30
III, x = 1	$(Pro)_2$	calcd	17.60	4.43	15.39	5.40	9.26
		obsd	18.19	4.89	15.48	5.50	9.80
IV, x = 2	$(Pro)_3$	calcd	20.90	4.76	15.09	4.88	8.38
		obsd	20.60	5.39	14.45		8.30
V, x = 1	$(Pro)_4$	calcd	24.28	4.86	15.25	4.58	7.86
		obsd	23.21	5.66	15.57	4.00	9.3

[a] iso = isonicotinoyl group.

intramolecular electron-transfer properties of these binuclear oligoproline complexes.

Experimental Section

The materials and methods used in this work have been fully described in ref 8. The synthesis of the binuclear Ru–(iso-$(Pro)_n$)–Co complexes is described in ref 8 and 9. The procedure for the kinetics experiments and the treatment of the data are described in ref 8. Initial rates were measured for the $[(NH_3)_5Co((Pro)_n\text{-iso})Ru(NH_3)_4OH_2]^{5+}$ complexes (n = 1–4) over a range of 1–10% extent of reaction. The reaction was then quenched by using excess ammonium persulfate, and the final absorbance

(1) (a) Rothe, M.; Theysohn, R.; Steffer, K. D.; Schneider, H.; Amani, M.; Kostrzewa, M. *Angew. Chem., Int. Ed. Engl.* **1970**, *8*, 919–20. (b) Rothe, M.; Rott, H. *Ibid.* **1976**, *15*, 770–1.
(2) (a) Chao, Y. H.; Bersohn, R. *Biopolymers* **1978**, *17*, 2761–7. (b) Chiu, H. C.; Bersohn, R. *Ibid.* **1977**, *16*, 277–88.
(3) (a) Grathwohl, C.; Wuthrich, K. *Biopolymers* **1976**, *15*, 2025–41. (b) Grathwohl, C.; Wuthrich, K. *Ibid.* **1976**, *15*, 2043–57.
(4) Deslauriers, R.; Becker, J.; Steinfield, A.; Naider, F. *Biopolymers* **1979**, *18*, 523–38.
(5) (a) Stryer, L. *Annu. Rev. Biochem.* **1978**, *47*, 319–46. (b) Stryer, L.; Haughland, R. P. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *58*, 719–26.
(6) Gabor, G. *Biopolymers* **1968**, *6*, 809–16.
(7) The distance is fixed for processes taking place at time scales faster than cis–trans proline isomerization (see ref 3).
(8) Isied, S.; Vassilian, A., *J. Am. Chem. Soc.*, preceding paper in this issue.
(9) Isied, S.; Vassilian, A.; Lyon, J. M. *J. Am. Chem. Soc.* **1982**, *104*, 3910–16.

Table II. Intramolecular Electron-Transfer Rates for the Proline Complexes at Different Temperatures

complex[a]	$10^5 k$,[b] s^{-1}			
	24.8 °C	29.0 °C	33.2 °C	37.7 °C
Co-(Pro-iso)-Ru	10.4	18.7	27.8	38.6
Co-((Pro)$_2$-iso)-Ru	0.64	1.15	1.93	2.39
	24.7 °C	29.5 °C	32.4 °C	35.2 °C
Co-((Pro)$_3$-iso)-Ru	5.59	8.73	11.5	13.0
	24.7 °C	29.5 °C	33.2 °C	35.2 °C
Co-((Pro)$_4$-iso)-Ru	13.7	19.0	23.1	24.4

[a] Co = $(NH_3)_5$Co-, Ru = $(OH_2)(NH_3)_4$Ru-, [Co^{III}-((Pro)$_n$-iso)-Ru^{III}] = 1×10^{-3}-5×10^{-4} M and [Co^{III}-((Pro)$_n$-iso)-Ru^{II}] = 5.7×10^{-5}-5.9×10^{-5} M in 1.0 M HTFA. [b] Each rate constant is the average of two different determinations.

was measured. The errors in the activaton parameters were calculated as in ref 8. The circular dichroism (CD) spectra were obtained on a Cary 60 spectrometer.

Results

A. Synthesis and Characterization of the Complexes. The series of cobalt–ruthenium binuclear complexes was synthesized by methods described previously.[8,9] The complexes were characterized by a number of physicochemical techniques. Table I summarizes the C, H, N, Co, and Ru elemental analyses on the purified complexes. The elution behavior on high-pressure liquid chromatography (reverse-phase C_{18} column) was used as a criterion of purity prior to elemental analysis. The top part of Figure 2 shows an increase in elution time with the oligomer size for [$(NH_3)_5$Co(Pro)$_n$] (n = 1–4). The same series after derivatization with the isonicotinoyl group is shown in the bottom portion of Figure 2. Also in this case retention time increases with the number of prolines in the oligomer. The visible absorption spectra of the Ru^{III}–(iso-(Pro)$_n$)–Co^{III} (n = 1–4) binuclear complexes showed the typical d–d band expected[10] for [$(NH_3)_5CoO_2CR$] (λ_{max} 502–503, $\epsilon \sim 80$ M^{-1} cm^{-1}). The other band (λ_{max} 350 nm) is masked by Ru(III) absorptions and is only seen as a shoulder. Reduction of the Ru(III) center to Ru(II) resulted in an intense band (λ_{max} 460 nm, $\epsilon \sim 0.80 \times 10^4$ M^{-1} cm^{-1}). No significant spectral differences were detected between the different oligoproline complexes.

The reduction potential of the Ru(III) site was determined by differential pulse polarography and cyclic voltammetry. Upon reduction of the Ru(III) center, two waves are observed in the cyclic voltammetry and in the differential pulse polarography. The first wave ($E_{1/2}$ = 0.34 V vs. NHE) appears in major proportions and corresponds to the reduction of the Ru center in [SO_4-$(NH_3)_4Ru^{III/II}$–(iso-(Pro)$_n$)–$Co(NH_3)_5$]. Oxidation of the Ru(II)–oligoproline complex shows only one band at $E_{1/2}$ = 0.43 V vs. NHE, corresponding to the oxidation of Ru in [OH_2-$(NH_3)_4Ru^{II/III}$–(iso-(Pro)$_n$)–$Co(NH_3)_5$]. Upon reduction, the sulfate is rapidly aquated ($t_{1/2} \sim 200$ ms) from the Ru^{II} complex,[11] resulting in the formation of *all-trans*-[$OH_2(NH_3)_4Ru^{II}$–(iso-(Pro)$_n$)–$Co(NH_3)_5$], which undergoes reversible oxidation–reduction, with $E_{1/2}$ = 0.43 V vs. NHE. The aquo species is the only species undergoing intramolecular electron transfer at the time scales involved.

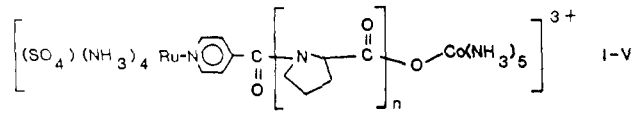
B. Electron-Transfer Kinetics. The details of the intramolecular electron-transfer experiment have been described elsewhere[8,9] and are summarized by eq 1 and 2. (For simplicity, the ammine ligands from the binuclear complexes have not been included.)

$$Ru^{III}\text{–L–}Co^{III} + [(NH_3)_6Ru]^{2+} \rightarrow Ru^{II}\text{–L–}Co^{III} + [(NH_3)_6Ru]^{3+} \quad (1)$$

$$Ru^{II}\text{–L–}Co^{III} \xrightarrow{k} Ru^{III}\text{–L} + Co^{2+} \quad (2)$$

L = bridging ligand

Table III. Intramolecular Electron-Transfer across Oligoprolines



n = 0, 1, 2, 3, 4

I–V

n	complex	$10^5 k$,[a] s^{-1}	$\Delta H^{\ddagger}$, kcal/mol	$\Delta S^{\ddagger}$, eu
0	Co-iso-Ru (I)[b]	1200	19.7 ± 0.2	−1.0 ± 0.5
1	Co-(Pro-iso)-Ru (II)	10.4	18.0 ± 1.1	−16 ± 4
2	Co-((Pro)$_2$-iso)-Ru (III)	0.64	18.6 ± 1.9	−20 ± 6
3	Co-((Pro)$_3$-iso)-Ru (IV)	5.6	14.5 ± 1.1	−29 ± 4
4	Co-((Pro)$_4$-iso)-Ru (V)	14.0	10.0 ± 0.7	−43 ± 3

[a] 24.8 ± 0.05 °C; 0.95 M HTFA. [b] Reference 22.

Table IV. Circular Dichroism Spectra (in the UV Region) for the [$(NH_3)_5$Co-((Pro)$_n$-iso)]$^{3+}$ Complexes (n = 1–4)[a]

complex	λ_{max}, nm ($\Delta\epsilon$)
[$(NH_3)_5$Co-(Pro-iso)]$^{3+}$	250 (−3.8), 190 (−7.2)
[$(NH_3)_5$Co-((Pro)$_2$-iso)]$^{3+}$	237 (−8.9), 192 (−7.8)
[$(NH_3)_5$Co-((Pro)$_3$-iso)]$^{3+}$	235 (−5.8), 198 (−32.1)
[$(NH_3)_5$Co-((Pro)$_4$-iso)]$^{3+}$	240 (−10.5), 202 (−48.8)

[a] All of the above CD spectra have negative elipticity [θ]; $\Delta\epsilon = \epsilon_L - \epsilon_R = [\theta]/3300$; θ in deg cm^2 $dmol^{-1}$.

The complexes were synthesized as Ru^{III}–L–Co^{III} salts. Selective reduction of the Co^{III}–L–Ru^{III} by [$(NH_3)_6Ru]^{2+}$ (and in some cases by Eu^{2+}) resulted in the formation of the binuclear precursor complex (eq 1). The rate of the intramolecular electron-transfer reaction (eq 2) was followed spectrophotometrically at λ_{max} 480 nm. The electron-transfer reaction (eq 2) followed first-order kinetics,[8] and no interference from bimolecular processes was observed at the concentrations used (2×10^{-5}–10×10^{-5} M). Table II reports the first-order rate constants at different temperatures. Table III shows a comparison of the rates (at 24.8 °C) and the activation parameters calculated from the data in Table III. For these slow reactions ($t_{1/2} \sim$ hours) the variation in the rate constants between the different determinations was 2–8%. The resulting errors in activation parameters are shown in Table III.

C. Circular Dichroism Studies. In the CD spectra the [$(NH_3)_5$Co-((Pro)$_n$-iso)] complexes showed broad, weak negative bands between 250 and 235 nm, followed by a much stronger band between 190 and 202 nm. Table IV presents the results for the series n = 1–4, showing the λ_{max} and $\Delta\epsilon$ of the bands.

Discussion

There are two areas of research on proline oligomers in the literature that are relevant to the subject of intramolecular electron transfer across polyprolines. The first is the information on cis–trans isomerism that proline rings undergo. Wuthrich et al.[3] showed that in acidic aqeuous solutions of (Ala-Pro)$_n$, the trans isomer predominates, while at higher pH (i.e., pH >2), a mixture of cis and trans isomers exists. Bersohn et al.[2,4] studied a series of (Pro)$_n$ oligomers by using ^1H and ^{13}C NMR. Their results show that in aqueous media the trans isomers predominate. Brandt et al.[12] have studied proline isomerization in peptides and proteins extensively. Proline isomerization seems to be one of the slowest steps (and therefore rate determining) in protein folding.[13] Brandt, Bovey, and others[3,12,14] have studied the rate of isomerization of cis–trans X–proline peptides in model compounds. A

(10) Gould, E. S.; Taube, H. *J. Am. Chem. Soc.* **1964**, *86*, 1318.
(11) (a) Isied, S.; Taube, H. *J. Am. Chem. Soc.* **1973**, *95*, 8198. (b) Isied, S., Ph.D. Thesis, Stanford University, 1974.
(12) (a) Lin, L.; Brandt, J. F. *Biochemistry* **1983**, *22*, 553–9. (b) Brandt, J. F.; Halvorson, H. R.; Brennan, M. *Ibid.* **1975**, *14*, 4953.
(13) For a review of proline isomerization, see: Kim, P. S.; Baldwin, R. L. *Annu. Rev. Biochem.* **1982**, *51*, 459–89.
(14) Cheng., H. N.; Bovey, F. A. *Biopolymers* **1977**, *16*, 1465–72.

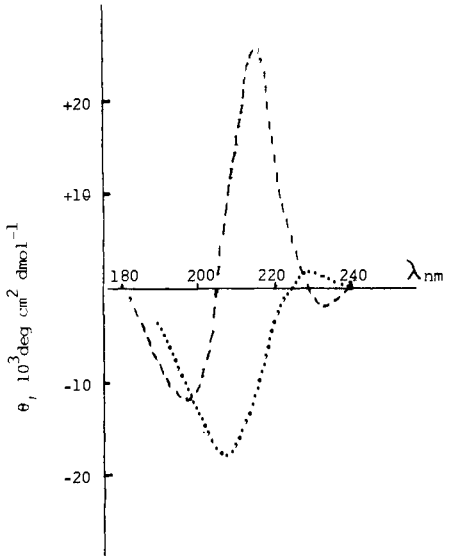


Figure 3. CD spectra of poly(proline I) (trans) (···) and poly(proline II) (cis) (---). (Reprinted with permission from Wiley, ref 15.)

$t_{1/2} \sim$ 1–2 min was observed for this process.

The second area of oligoproline research related to our studies is their use as spacers in energy-transfer studies. Stryer and others studied the excitation energy transfer across a series of oligoproline residues that have been modified with donor–acceptor groups.[5,6] They found that oligoprolines D–$(Pro)_n$–A (n = 5–12) (D and A are donor and acceptor) exist in the trans conformation in ethanol, with the prolines acting as a spacer of defined length between the donor and acceptor chromophores.[5,6] Bersohn et al.[2] studied the energy-transfer properties of Trp$(Pro)_n$*Tyr* (n = 0–5), where tyrosine and tryptophan amino acids are attached to the C and N terminals of oligoproline, respectively. Their conclusion was that in these molecules the prolines exist in a mixture of cis and trans isomers. The underivatized oligoprolines, however (studied by the same authors), were found to be predominantly (>90%) in the trans conformation. An equilibrium constant ≥10 favoring the trans conformation can be estimated for a single proline peptide bond in aqueous acidic media.

Our studies were conducted in aqueous acidic media, which favors the formation of the *trans*-proline isomers.[3] The CD results on the $[(NH_3)_5Co$–$((Pro)_n$-iso)] complexes (Table IV) are only slightly different from those reported in the literature for the proline peptide oligomers.[1] For triproline, a weak positive and a strong negative Cotton effect are observed by bands at 226 and 197 nm.[1] For the cobalt–triproline complex, $[(NH_3)_5Co$–$((Pro)_3$-iso)], a single strong band is observed at λ = 197 nm, which is absent for n = 1 and 2. The appearance of this band in the cobalt–triproline complex is a strong indication that secondary structure starts with the triproline complex and is in agreement with previous work on oligoprolines.[1] The far-UV band maximum at λ 197 nm in the cobalt–triproline complex shifts to 202 nm for the $[(NH_3)_5Co$–$((Pro)_4$-iso)] oligomer, a shift to higher wavelength for increasing n, in agreement with the work on proline peptide oligomers.[1] The far-UV band for the cobalt–proline oligomer complexes is very similar to that for poly(proline II), the all-trans conformation. The reported CD spectra of poly(proline I) (cis) and poly(proline II) (trans) are shown in Figure 3.[15]

The variation in the rate of intramolecular electron transfer in these Co–$((Pro)_n$-iso)-Ru complexes (n = 1–4) is found in Table III. In proceeding from zero to one proline bridge in compound I to compound II, we observe a significant decrease in rate (factor of 120). This is attributed to the increase in distance, ~3 Å for the proline residue, between the donor and acceptor. (The trans conformation of these oligoprolines implies that the cobalt and ruthenium metal ions are separated by multiples of 3.1 Å per proline residue.[2]) A further decrease in rate (a factor of 15) was observed between compounds II and III with one and two prolines.

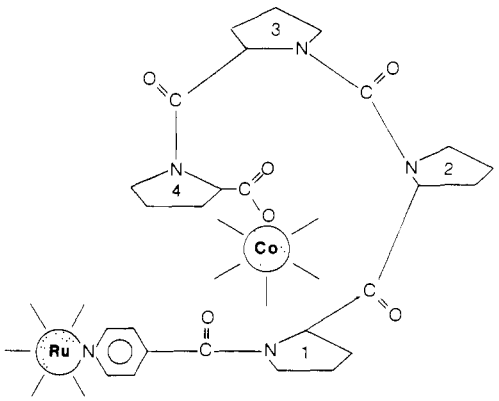


Figure 4. Proposed cis conformation of $[(NH_3)_5Co$–$((Pro)_4$-iso)–Ru–$(NH_3)_4OH_2]^{5+}$ in which electron transfer takes place. (Adapted from ref 21.)

With three and four prolines separating the donor and acceptor, further decreases in rate are expected when the prolines are in the trans extended form. However, the results show an order-of-magnitude *increase* in rate for three prolines (n = 3) over that for two prolines (n = 2). With four prolines (n = 4) a further slight increase in rate is also observed. The observed rate constants, which decrease for n = 1 and 2 and then increase for n = 3 and 4 as the expected distance between the metal ion centers increases, can only be understood if one examines the dynamics of trans proline isomerization.[12,14] On the basis of studies of a large number of proline peptides, the rotation around one proline–peptide bond is known to occur with a half-life of approximately 1–2 min, with a $\Delta H^{\ddagger}$ of 18–20 kcal/mol and a $\Delta S^{\ddagger} \sim 0$. Because the rate of electron transfer in this series of complexes is slow ($t_{1/2} \sim$ several hours), there is enough time for proline isomerization to occur and bring the donor and acceptor into closer proximity.[16] On this long time scale for electron transfer, proline can isomerize prior to electron transfer. Thus, we propose that the species undergoing electron transfer is in a cis conformation, where the three or four prolines in the bridge are all cis to one another. Figure 4 shows how close the cobalt and ruthenium get when the tetraproline is in a completely cis conformation. Note that only a small amount of cis proline is required for the electron-transfer reaction. The same rationale applies for the bridging triproline case, except that the metal ions are slightly further apart.[17]

A number of studies have been published on the rates of intramolecular electron transfer across different bridging groups using ruthenium and cobalt donors and acceptors.[11,18] The uniqueness of the present study lies in using a peptide bridging ligand and systematically extending it by one amino acid residue at a time, keeping all other factors the same. Four proline residues have been introduced one at a time, and their effect on the rate of intramolecular electron transfer has been evaluated. The rate constant for the intramolecular electron transfer decreases by 2000 times when the Co^{III}–bridge–Ru^{II} is bridged by two prolines moieties, compared to no prolines in the bridge. This decrease in rate occurs even though there is no change in the driving force of the reaction. The decrease in rate between n = 0 and n = 2

(15) (a) Ronish, E. W.; Krimm, S. *Biopolymers* **1974**, *13*, 1635–51. (b) Helbecque, N.; Loucheux-Lefebvre, M. H. *Int. J. Pept. Protein Res.* **1982**, *19*, 94–101.

(16) For the n = 3 and n = 4 proline complexes (Figure 4), the half-time required to assemble the isomer, which undergoes electron transfer, can be approximated as $2^n t_{1/2}$ min (n = is the number of prolines and $t_{1/2}$ is the half-time of isomerization of a single proline). This expression will hold if every proline isomerization is independent of the others in the chain.

(17) For a rate-limiting electron-transfer process, the rate constant for the n = 3 and n = 4 prolines could be corrected for the equilibrium constant of the cis–trans isomerization. This correction would be valid if electron transfer proceeds in only one conformation (e.g., the all cis) with a known equilibrium constant. Since the equilibrium constant is unknown at this time, no correction has been made.

(18) Taube, H. In "Tunneling in Biological Systems"; Chance, B., DeVault, D., Frauenfelder, H., Marcus, R., Schrieffer, J., Sutin, N., Ed.; Academic Press: New York, 1979.

results from an increase in the separation of the donor and acceptor by ~6 Å. The largest drop in rate occurs when the first proline is added (factor of >100). The separation between the donor and acceptor increased by ~3 Å. This large decrease in rate is attributed to an increase in the outer-sphere reorganizational energy and a decrease in the electronic coupling factor,[19] with the latter predominating. For cases where the distances are fixed during the electron-transfer process, quantitative estimates for the decrease in rate could be obtained. However, because the rate of electron transfer is slow compared to proline isomerization in this study, quantification of the data as a function of distance cannot be done.

When $[(NH_3)_5Os^{II}-]$ is used in place of $[H_2O(NH_3)_4Ru^{III}-]$ as a donor, the driving force for the intramolecular electron-transfer reaction can be increased by about 0.6-0.7 V.[20] This is expected to increase the rate of electron transfer by 5 orders of magnitude ($t_{1/2} < 1$ s).[20] For such a fast reaction the question is whether the intramolecular electron-transfer reaction will take place at the long distance of the fully trans isomer or whether electron transfer will wait for the cis-trans isomerization to occur ($t_{1/2} \sim$ 1-2 min), to bring the donor and acceptor into close proximity. Furthermore, with these fast rates proline oligomers will be rigid spacers, thus allowing quantification of the decrease in rate of electron transfer with distance. The osmium(II)-cobalt(III) proline series is currently being investigated to address these issues.[20]

Acknowledgment. This work was supported by the National Institutes of Health (Grant GM 26324). S.I. is the recipient of a National Institutes of Health Career Development Award (AM 00734) (1980-1985) and a Camille and Henry Dreyfus Teacher-Scholar Award.

Registry No. $[SO_4(NH_3)_4Ru-(iso-(Pro))-Co(NH_3)_5](BF_4)_3 \cdot HBF_4$, 88510-36-5; $[SO_4(NH_3)_4Ru-(iso-(Pro)_2)-Co(NH_3)_5](BF_4)_3 \cdot HBF_4$, 88524-99-6; $[SO_4(NH_3)_4Ru-(iso-(Pro)_3)-Co(NH_3)_5](BF_4)_3 \cdot HBF_4$, 88525-02-4; $[SO_4(NH_3)_4Ru-(iso-(Pro)_4)-Co(NH_3)_5](BF_4)_3 \cdot HBF_4$, 88525-05-7; $[(NH_3)_5CoPro]^{2+}$, 68582-22-9; $[(NH_3)_5Co(Pro)_2]^{2+}$, 68582-28-5; $[(NH_3)_5Co(Pro)_3]^{2+}$, 88525-06-8; $[(NH_3)_5Co(Pro)_4]^{2+}$, 68582-19-4; $[(NH_3)_5CoPro\text{-}iso]^{2+}$, 88496-37-1; $[(NH_3)_5Co(Pro)_2\text{-}iso]^{2+}$, 88496-39-3; $[(NH_3)_5Co(Pro)_3\text{-}iso]^{2+}$, 88525-07-9; $[(NH_3)_5Co(Pro)_4\text{-}iso]^{2+}$, 88525-08-0.

(19) (a) Sutin, N. *Acc. Chem. Res.* **1982**, *15*, 275-82. (b) Sutin, N. In ref 18.
(20) Vassilian, A.; Magnuson, R.; Schwarz, H.; Isied, S., work in progress.
(21) Traub, W.; Shmueli, U. *Nature (London)* **1963**, *198*, 1165.
(22) Zawacky, S.; Taube, H. *J. Am. Chem. Soc.* **1981**, *103*, 3379-87.
(23) Stryer, L. "Biochemistry"; W. H. Freeman: San Francisco, 1981.

Photochemistry of 2-Phenyl-1,2-dihydronaphthalene. A Competition between a Singlet State Di-π-methane Rearrangement and a Ring-Opening Reaction

Joseph J. M. Lamberts and Wim H. Laarhoven*

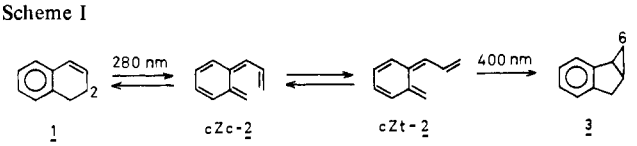
Contribution from the Department of Organic Chemistry, Catholic University, Toernooiveld, 6525 ED Nijmegen, The Netherlands. *Received January 26, 1983*

Abstract: Irradiation of 2-phenyl-1,2-dihydronaphthalene (**4**) at 300 nm in hexane leads to three primary photoproducts: *endo*- and *exo*-6-phenylbenzobicyclo[3.1.0]hex-2-ene (*endo*-**5** and *exo*-**5**) and 7,12-dihydrodibenzo[*a*,*d*]cyclooctatetraene (**6**). Irradiation of labeled **4** (**4**-*3*,*4*-d_2) showed that **5** arises via a di-π-methane rearrangement as well as via electrocyclic ring opening, single bond rotation, and photocycloaddition. The di-π-methane rearrangement proceeds partly as a triplet reaction, giving both epimers of **5**, but mainly as a stereoselective singlet reaction, giving only *endo*-**5**. The pathway via ring opening and cycloaddition yields *exo*-**5**, which arises from that conformer of **4** having the phenyl group in the pseudoequatorial position (PE-**4**). The other conformer (PA-**4**) yields mainly **6** (Scheme VI) because the ring opening can be followed by an 8π-electrocyclic ring closure. On irradiation of **4** at 254 nm the stereoselectivity of the singlet reactions is less clear, because *endo*-**5** and *exo*-**5** are interconverted. Parallel with the interconversion 2-benzylideneindan (**7**) is formed as a secondary photoproduct under these conditions.

The photochemistry of 1,2-dihydronaphthalenes has been studied for over 10 years. In 1971 Salisbury[1] proposed the reaction mechanism, given in Scheme I for the conversion of the parent compound (**1**) into benzobicyclo[3.1.0]hex-2-ene (**3**). It was based on the observation that a broad spectrum of wavelengths was needed to bring about the conversion. A long-wavelength absorption band, corresponding to the supposed pentaene intermediate (*cZc*-**2**) has actually been observed in low-temperature irradiations of some 1,2-dihydronaphthalene derivatives.[2,3]

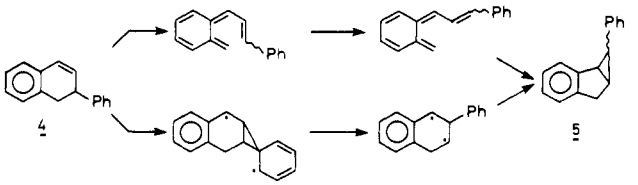
We anticipated that 2-phenyl-1,2-dihydronaphthalene (**4**) might be a derivative of **1** of special interest in photochemical studies, because it can also be conceived as a 3-phenylpropene able to undergo a di-π-methane rearrangement. The reaction mechanism of the basic di-π-methane rearrangement is given in Scheme II in terms of intermediate diradical structures.[4] Recently, indications for the existence of such intermediates have been found[5], but concerted reactions of this kind cannot be excluded.

Scheme I



Scheme II

Scheme III



(1) K. Salisbury, *Tetrahedron Lett.* 737 (1971).
(2) H. Kleinhuis, R. L. C. Wijting, and E. Havinga, *Tetrahedron Lett.* 255 (1971).
(3) U. Widmer, H. Heimgartner, and H. Schmid, *Helv. Chim. Acta* **58**, 2210 (1975).

0002-7863/84/1506-1736$01.50/0