September, 1989] *Bull. Chem. Soc. Jpn.,* ***62****, 2897—2901 (1989)*

Racemization of Phenylalanine Coordinated to Bis(2,2′-bipyridine)ruthenium(II) Complexes in Basic Aqueous Solutions

Takashi Nagai
Laboratory of Chemistry, Nippon Medical School, Kosugi-chou, Nakahara-ku, Kawasaki 211

(Received January 31, 1989)

Racemization of the phenylalaninato ligand in Λ- and Δ-[Ru(phe)(bpy)$_2$]$^+$ (phe; phenylalaninate anion, bpy; 2,2′-bipyridine) complexes was found in basic aqueous solutions. It was proceeding without conformational change around the ruthenium ion in the dark. The equilibrium and rate constants of the racemization were determined in basic solutions at 100 °C. The reaction was base-catalyzed and not affected by photoexcitation of the complex ions.

The photochemical inversion of diastereoisomers of [Ru(*S*-am)(bpy)$_2$](ClO$_4$)$_n$ (am; amino acidate anion) has been studied by Vagg et al.[1–5] They have reported that in aqueous solutions these complexes are photolabile and the inversion reaction around the metal ion occurs on irradiation of light. By contrast, these complexes are markedly stable in darkness: No inversion occurs even on boiling the complexes in aqueous solutions for a few hours. However, we found that the coordinated phenylalanine in [Ru(phe)(bpy)$_2$]$^+$ ion was gradually racemized in basic solutions in the dark. The rate and equilibrium constants of the racemization at 100 °C were determined by the use of the chromatographic method. Moreover, effects of photoexcitation of the complexes on the racemization were examined by light irradiation during the reaction.

Experimental

All materials used were of reagent grade, and used without further purification. The UV-visible spectra were obtained with a HITACHI 340 spectrophotometer. Circular dichroism (CD) spectra were obtained using a JASCO J-500 spectrophotometer.

Λ- and Δ-[Ru(*S*-phe)(bpy)$_2$]ClO$_4$ Hydrates. The mixture of Λ- and Δ-isomers was prepared and partially resolved by Vagg et al.[1,4] However, we prepared it by a different way and resolved it completely to obtain perchlorates of the Λ- and Δ-forms.

cis-[Ru(CO$_3$)(bpy)$_2$]·3H$_2$O[6] (1 g) was dissolved in 200 cm^3 of hot water, and (*S*)-phenylalanine (0.4 g) was added. The solution was stirred at 50 °C for 25 min in the dark. The deep-red solution was cooled, and then added on a short column (12.5 cm height×2.5 cm diameter) of SE-Toyopearl.[7] The adsorbed complexes were eluted with 0.2 mol dm^{-3} Na$_2$SO$_4$ to remove minor impurities. The main adsorption band was eluted, and the complexes in the eluate were precipitated by adding an excess of NaClO$_4$. The dark-red precipitate was filtered, washed with a small amount of water, and air-dried (yield 1.2 g).

The precipitate (0.7 g) was dissolved in 400 cm^3 of water, and placed on a column (56 cm height×2.6 cm diameter) of SE-Toyopearl. The adsorbed complex cations were washed with water and then eluted with 0.05 mol dm^{-3} sodium (*R*,*R*)-tartrate to develop two adsorption bands by the recycle chromatographic technique. The two bands were eluted separately, and each eluate was evaporated to about one-tenth of the initial volume by a rotary evaporator at about 40 °C, and then methanol was added to remove sodium (*R*,*R*)-tartrate. The filtrates were evaporated to take off the methanol, and dark-red crystals were precipitated by adding NaClO$_4$. The products were filtered off, washed with a small amount of water and dried under vacuum. All the above procedures were performed in the dark. The assignment of the absolute configuration of the complexes was determined by the sign of the peaks at about 290 nm of the CD spectrum in accordance with Vagg's report.[1] The products from the first and second adsorption bands were assigned to Δ- and Λ-isomers, respectively, and were used as the standard samples for measuring absorption and CD spectra in kinetic experiments.

Δ-Isomer. Found: Ru, 15.10; C, 49.04; H, 3.76; N, 9.86%. Calcd for Δ-[Ru(*S*-phe)(bpy)$_2$]ClO$_4$·H$_2$O: Ru, 14.54; C, 50.11; H, 4.06; N, 10.08%. ABS (H$_2$O) 492 nm (ε 9250); CD (H$_2$O) 350 nm ($\Delta\varepsilon -13.7$). Λ-Isomer. Found: Ru, 13.60; C, 46.81; H, 3.57; N, 9.50%. Calcd for Λ-[Ru(*S*-phe)(bpy)$_2$]ClO$_4$·3H$_2$O: Ru, 13.82; C, 47.64; H, 4.41; N, 9.58%. ABS (H$_2$O) 492 nm (ε 9250); CD (H$_2$O) 350 nm ($\Delta\varepsilon$ 16.1).

Starting Materials of Kinetic Experiments. A solution containing *cis*-[Ru(CO$_3$)(bpy)$_2$] and (*S*)-phenylalanine was refluxed for 20 min at 100 °C. After cooling, a dark-red product was precipitated by adding NaClO$_4$. The product was identified as [Ru(phe)(bpy)$_2$]ClO$_4$·2H$_2$O by the spectrophotometric methods, and it was found by the chromatographic separation that the product comprised four isomers which consisted of two predominant ones, Λ- and Δ-[Ru(*S*-phe)(bpy)$_2$]ClO$_4$·2H$_2$O, and two minor ones, Λ- and Δ-[Ru(*R*-phe)(bpy)$_2$]ClO$_4$·2H$_2$O, because of partial racemization of the coordinated phenylalanine.[8] This product was used as starting materials in the following experiments.

Rate Determination Procedures. The starting materials, [Ru(phe)(bpy)$_2$]ClO$_4$·2H$_2$O, of 0.2—0.25 mmol were dissolved in 100 cm^3 of a refluxing Sørensen buffer solution (0.05 mol dm^{-3} borax–0.1 mol dm^{-3} NaOH) at (100±2) °C in an atmosphere of nitrogen in darkness. In the case of a typical experiment, a 10 cm^3 aliquot of the reaction solution was taken out after 1 and 2 hours later and cooled in ice. The solution was added on a column (27 cm height×2 cm diameter) of SE-Toyopearl. The adsorbed complex cations were eluted with 0.1 mol dm^{-3} sodium (*R*,*R*)-tartrate, and completely resolved into two isomeric groups. One group contained Δ-[Ru(*S*-phe)(bpy)$_2$]$^+$ (Δ-*S*) and Λ-[Ru(*R*-phe)(bpy)$_2$]$^+$ (Λ-*R*) ions which were first eluted, and another contained Λ-[Ru(*S*-phe)(bpy)$_2$]$^+$ (Λ-*S*) and Δ-[Ru(*R*-phe)-

$(bpy)_2]^+$ (Δ-R) ions which were secondly eluted. Decomposition products were negligible. An absorbance at 492 nm (ε=9250 for all of four isomers) gave the total concentration of the complexes, and a circular dichroism at 350 nm ($\Delta\varepsilon=-13.7$ for Δ-S, 16.1 for Λ-S, 13.7 for Λ-R, and −16.1 for Δ-R) reflected a ratio of the concentrations between two enantiomers in each eluate. The fractions of each isomer to the total complex ions were calculated from these spectrophotometric data and volumetric ratio of the two eluates as shown in Table 1. The data at t=0 were obtained from the chromatographic separation of the starting complex solution which was not refluxed. The data at equilibria were obtained from the refluxing solutions at pH 9.03 and 8.64 for 10 and 45 h, respectively. These reaction periods were more than ten times of half-lives estimated with effective rate constants (vide infra).

As regards racemization of the coordinated phenylalanine, the pseudo first-order rate constants, k_{AD}, k_{BD}, k_{AL}, and k_{BL}, were defined as follows:

$$\Delta\text{-}S \underset{k_{BD}}{\overset{k_{AD}}{\rightleftarrows}} \Delta\text{-}R, \tag{1}$$

$$\Lambda\text{-}R \underset{k_{BL}}{\overset{k_{AL}}{\rightleftarrows}} \Lambda\text{-}S. \tag{2}$$

In the present experiments, the fractions of the four isomers were obtained for each reaction time, and hence two integrated rate equations were yielded independently from both rates of disappearance of the reactant and appearance of the product with regard to one of the reversible reactions. The two expressions for the effective rate constant, k_{eff}, defined as sum of the rate constants for the forward and reverse directions of reaction (1), are

$$k_{eff} = \frac{1}{t}\cdot\ln\left(\frac{P[\Delta\text{-}S]_0 - P[\Delta\text{-}S]_e}{P[\Delta\text{-}S]_t - P[\Delta\text{-}S]_e}\right) \tag{3}$$

and

$$k_{eff} = \frac{1}{t}\cdot\ln\left(\frac{P[\Delta\text{-}R]_0 - P[\Delta\text{-}R]_e}{P[\Delta\text{-}R]_t - P[\Delta\text{-}R]_e}\right), \tag{4}$$

where $P[A]$ is the fraction of A, and subscripts 0 and e refer to zero time and equilibrium conditions, respectively. The values evaluated by the two equations almost agreed with each other, and thereupon the mean value was adopted as k_{eff}. The rate constants, k_{AD} and k_{BD}, were calculated by

$$k_{eff} = k_{AD} + k_{BD} = k_{BD}\cdot(1 + K), \tag{5}$$

where K is the equilibrium constant defined as

$$K = P[\Delta\text{-}R]_e/P[\Delta\text{-}S]_e = P[\Lambda\text{-}S]_e/P[\Lambda\text{-}R]_e. \tag{6}$$

The each value of the fractions at equilibria ($P[\Delta\text{-}S]_e$, $P[\Delta\text{-}R]_e$, $P[\Lambda\text{-}S]_e$, and $P[\Lambda\text{-}R]_e$) was assumed to be the same for all runs at various pHs. The mean values of the corresponding fractions at pH 8.64 and 9.03 were adopted as the values at equilibria, and used to calculate the rate and equilibrium constants. The other rate constants, k_{AL} and k_{BL}, were similarly derived by using the fractions of the isomers in reaction (2). The values of each rate constant were calculated corresponding to two reaction times other than at equilibrium, and they were the same at respective pH values. The mean observed values of k_{AD}, k_{BD}, k_{AL}, and k_{BL} are summarized in Table 2.

The pH values at the reaction temperature, (100±2) °C, were estimated by extrapolation of the pH values measured at room temperature to those at 100 °C by reference to the temperature dependence data of pH of Sørensen buffer solution.

Experiments on effects of photoexcitation were carried out at pH 9.03. Light from 300 W short-arc type Xe lamp was focused on an area of about 4 cm diameter, and irradiated to the refluxing solution through a Pyrex glass vessel.

Results and Discussion

Table 1 shows that the (S)-phenylalaninato complexes, Δ-S and Λ-S, were predominant components of the starting materials. As the racemization of the coordinated phenylalanine proceeded, the fractions of the (R)-phenylalaninato complexes, $P[\Delta\text{-}R]$ and $P[\Lambda\text{-}R]$, increased. From Table 1, it is also seen that the fraction of Δ-isomers, $P[\Delta]$, or that of Λ-ones, $P[\Lambda]$, was always constant for all the dark reactions. Preliminary experiments revealed that no inversion around the metal ion was detected even though an isomer was refluxed in a neutral aqueous solution for four hours in the dark, and the results were the same with those by Vagg et al.⁵⁾ From such observations, we assumed the dark reaction paths as illustrated in Scheme 1.

Scheme 1 expresses that racemization occurs only on the amino carboxylato ligand (paths I and II), while the paths of the inversion reaction (paths III and IV) are intercepted by some energy barriers. Absence of the reactions through the paths III and IV was supported by the fact that both fractions of Δ- and Λ-isomers, $P[\Delta]$ and $P[\Lambda]$, were maintained constant in addition to the total concentration of the complexes. The enantiomers, Δ-S and Λ-R or Δ-R and Λ-S, are energetically equivalent. At equilibria the ratios of the fractions of the enantiomers, ($P[\Delta\text{-}S]_e/P[\Lambda\text{-}R]_e$) and ($P[\Delta\text{-}R]_e/P[\Lambda\text{-}S]_e$), were not unity but equal to ($P[\Delta]/P[\Lambda]$), which was always constant at 0.92±0.01 for the dark reactions. This is also explainable as a result of absence of the paths III and IV.

For each pH, the values of k_{AD} and k_{BD} agree with k_{AL} and k_{BL}, respectively, within experimental errors as shown in Table 2. Hence it is confirmed that the paths I and II are statically and dynamically identical reactions. Then the pseudo first-order rate constants k_A will be used for k_{AD} and k_{AL}, and k_B for k_{BD} and k_{BL} in the following. Since the stability of the two isomers in path I or II differed from each other, the magnitude of k_A did not coincide with that of k_B in each pH. It is suggested that Λ-S (or Δ-R) is more stable than Δ-S (or Λ-R), because k_A is greater than k_B. This is consistent with Vagg's report.⁴,⁵⁾ He has described that the main

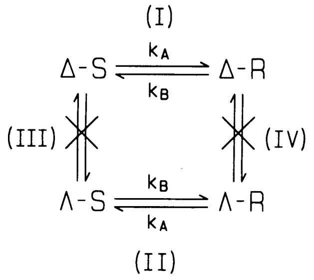
Table 1. Fractions of the Isomers during the Racemization at 100 °C

pH$^{a)}$	t/min	$P[\Delta\text{-}S]^{b)}$	$P[\Delta\text{-}R]$	$P[\Lambda\text{-}S]$	$P[\Lambda\text{-}R]$	$P[\Delta]^{c)}$	$P[\Lambda]$
—	0	0.440	0.043	0.490	0.027	0.483	0.517
—	0	0.445	0.041	0.492	0.023	0.486	0.515
8.25	122	0.407	0.072	0.470	0.051	0.479	0.521
	241	0.382	0.097	0.462	0.060	0.479	0.522
8.64	60	0.403	0.078	0.468	0.051	0.481	0.519
	120	0.366	0.113	0.451	0.070	0.479	0.521
	2700(∞)	0.169	0.312	0.340	0.180	0.481	0.520
9.03	60	0.288	0.188	0.410	0.114	0.476	0.524
	120	0.220	0.257	0.371	0.152	0.477	0.523
	600(∞)	0.166	0.315	0.339	0.180	0.481	0.519
9.24	60	0.241	0.240	0.376	0.143	0.481	0.519
	120	0.189	0.287	0.349	0.175	0.476	0.524
9.69	30	0.219	0.257	0.361	0.163	0.476	0.524
	60	0.179	0.298	0.346	0.177	0.477	0.523
9.03$^{d)}$	60	0.258	0.177	0.457	0.108	0.435	0.565
	120	0.223	0.246	0.381	0.150	0.469	0.531

a) Extrapolated values at 100 °C. b) Fraction of $[\Delta\text{-}S]$ to the total concentration of the complex ions. c) Fraction of $([\Delta\text{-}S]+[\Delta\text{-}R])$ to the total concentration of the complex ions. The mean value of $P[\Delta]$ is 0.479 ± 0.003, and that of $P[\Lambda]$ 0.521 ± 0.003 for the dark reactions. d) Under irradiation of Xe light.

Table 2. Racemization Rate Constants/10^{-5} s^{-1} at 100 °C

pH	k_{AD}	k_{AL}	k_{BD}	k_{BL}
8.25	1.1±0.1	1.3±0.3	0.59±0.05	0.68±0.02
8.64	2.8±0.2	3.0±0.2	1.5 ±0.1	1.6 ±0.1
9.03	15 ±1	15 ±1	7.7 ±0.3	7.9 ±0.5
9.24	23 ±1	27 ±3	12 ±1	14 ±1
9.69	57 ±4	70 ±10	30 ±2	37 ±5



Scheme 1. Reaction paths for the dark system.

factor to determining the stability of these complex species is the steric interaction between one of the bipyridine protons and amino acid side chain.

In Table 2, the rate constants at pH 9.03 are greater than the corresponding ones at pH 8.64 by a factor about 5. On the other hand, in Table 1, the equilibrium constants evaluated by Eq. 6 for these pH values are the same (K=1.9) and independent of pH. These results indicate that this is base-catalyzed reaction. Logarithms of k_{A} and k_{B} are plotted against pH in Fig. 1. The plots are linear and both slopes can be regarded as unity, and it is indicated that the racemization rates are the first-order with respect to OH$^-$ concentration. Hence, the expressions for the rate law are

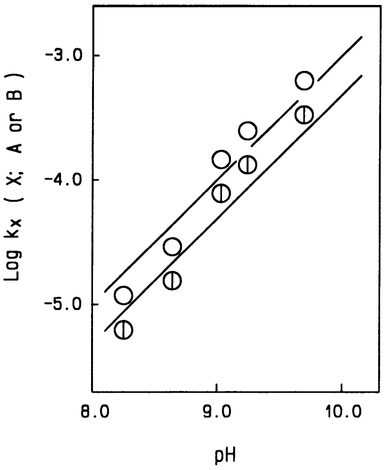


Fig. 1. Logarithms of the racemization rate constants k_{A} (○) and k_{B} (⦶) vs. pH. Lines are drawn with slope of 1.0.

Table 3. Reactions at Room Temperature (23—24 °C)

	$P[\Delta\text{-}S]$	$P[\Delta\text{-}R]$	$P[\Lambda\text{-}S]$	$P[\Lambda\text{-}R]$	$P[S]$	$P[R]$
Irradiated	0.383	0.061	0.510	0.045	0.894	0.106
Nonirradiated	0.420	0.062	0.475	0.043	0.895	0.105

pH of the solution: 10.70; The reaction time: 2.3 h for both the irradiated and the nonirradiated reactions.

$$R_A = k_A'[OH^-][\Delta\text{-}S] = k_A'[OH^-][\Lambda\text{-}R] \tag{7}$$

and

$$R_B = k_B'[OH^-][\Lambda\text{-}S] = k_B'[OH^-][\Delta\text{-}R], \tag{8}$$

where R_A and R_B represent the rates for the faster and slower reactions, respectively, and k_A' and k_B' are the corresponding second-order rate constants. The values of k_A' and k_B' are derived from k_A and k_B,[9] as follows:

$$k_A' = (0.19 \pm 0.07)\,\text{mol}^{-1}\cdot\text{dm}^3\cdot\text{s}^{-1},$$

$$k_B' = (0.10 \pm 0.04)\,\text{mol}^{-1}\cdot\text{dm}^3\cdot\text{s}^{-1} \quad (\text{at } 100\,^\circ\text{C}).$$

Bada[11] has determined the racemization rate of free phenylalanine at pH 7.6:

$$\log k(\text{year}^{-1}) = 17.05 - 6208/T.$$

When T is 373 K, k is evaluated to $8.1\times10^{-8}\,\text{s}^{-1}$. On the other hand, extrapolation of the present data to pH 7.6 gives $k_A \simeq 4.4\times10^{-6}\,\text{s}^{-1}$ and $k_B \simeq 2.3\times10^{-6}\,\text{s}^{-1}$. These values of k_A and k_B are ca. 50 and ca. 30 times greater than the rate constant of free phenylalanine, respectively.

Widely, the rate of racemization of amino acids chelated to a metal ion or in proteins and peptides are of the first-order with respect to hydroxide ion concentration, while the free amino acids racemize with a small pH dependence in basic solutions.[10—12] Accordingly, the present results suggest that the coordinated phenylalanine racemizes without breaking bonds to the ruthenium ion. As for reaction mechanism, it would be the same as generally recognized base-catalyzed one: α-proton is abstracted by hydroxide ion to form a planar carbanion intermediate.[10—15]

When light was irradiated on the reaction system, the rapid reaction paths III and IV in Sheme 1 were induced. On the assumption that the equilibria for paths III and IV are established rapidly enough as compared to paths I and II, the system is apparently written by

$$S_{\text{total}} \underset{k_{\text{ap}}}{\overset{k_{\text{ap}}}{\rightleftarrows}} R_{\text{total}}, \tag{9}$$

where S_{total} refers ($\Delta\text{-}S+\Lambda\text{-}S$) and R_{total} does ($\Delta\text{-}R+\Lambda\text{-}R$), and k_{ap} is the apparent first-order rate constant. If the light has no effect on the paths I and II, k_{ap} can be yielded by using k_A and k_B for the dark reaction system. The relationship is expressed by

$$k_{\text{ap}} = (k_A + k_B\cdot K_{\text{inv}})/(K_{\text{inv}} + 1), \tag{10}$$

where K_{inv} is equilibrium constant for the inversion reaction paths III and IV and defined as

$$K_{\text{inv}} = P[\Lambda\text{-}S]_e/P[\Delta\text{-}S]_e = P[\Delta\text{-}R]_e/P[\Lambda\text{-}R]_e. \tag{11}$$

Considering energetic relationship of the isomers, K_{inv} is essentially the same with K for the racemization of the coordinated amino acid. The values of the rate constants of the dark reaction under pH 9.03, $k_A = 1.5\times10^{-4}\,\text{s}^{-1}$ and $k_B = 7.8\times10^{-5}\,\text{s}^{-1}$, and equilibrium constant, $K_{\text{inv}} = K = 1.9$, are substituted in Eq. 10 to yield $1.0\times10^{-4}\,\text{s}^{-1}$ for k_{ap}. On the other hand, k_{ap} was determined to be $9.9\times10^{-5}\,\text{s}^{-1}$ by using data under irradiation of light in Table 1. The two values agree very well with each other.

Effects of the irradiation on the racemization were also examined with solutions at pH 10.70 at room temperature where no racemization was expected to take place in the dark. Results in Table 3 show that the racemization is not caused even by the irradiation, although the inversion around the metal ion occured.

It is concluded that the racemization of the coordinated amino acid proceeds independently of the photoexcitation of the complex ions. Commonly, the racemization rate of an amino acid increases with increasing stability of the carbanion intermediate. The extent of the stability is influenced greatly by the electron-withdrawing power of the metal ion in complexes, when amino acid is chelated as a bidentate.[10,15] Photoexcited states of complexes such as ones studied have been considered to be ^{3}(MLCT) and (dd) excited states[16] which bring some electron deficient state on the central metal ion and some distortion on metal–ligand bonds, respectively. The excited states cause secondary reactions such as the photosensitized one and ligand substitutions. However, the present studies indicate that effects of the photoexcited states of the complexes are limited in the bis(2,2′-bipyridine)ruthenium moiety and do not extend to the α-carbon of the amino carboxylato ligand or events concerning the excited states are much faster than the racemization.

References

1) R. S. Vagg and P. A. Williams, *Inorg. Chim. Acta,* **51**, 61 (1981).

2) R. S. Vagg and P. A. Williams, *Inorg. Chem.,* **22**, 355 (1983).

3) F. S. Stephens, R. S. Vagg, and P. A. Williams, *Inorg. Chim. Acta,* **72**, 253 (1983).

4) T. J. Goodwin, P. A. Williams, and R. S. Vagg, *Inorg. Chim. Acta,* **83**, 1 (1984).

5) R. S. Vagg, *J. Proc. Roy. Soc. N. S. Wales,* **117**, 99 (1984).

6) E. C. Johnson, B. P. Sullivan, D. J. Salmon, S. A. Adeyemi, and T. J. Meyer, *Inorg. Chem.,* **17**, 2211 (1978); M. Tanaka, T. Nagai, E. Miki, K. Mizumachi, and T. Ishimori, *Nippon Kagaku Kaishi,* **1979**, 1112.

7) M. Fujita, Y. Yoshikawa, and H. Yamatera, *Chem. Lett.,* **1982**, 437.

8) When a mixed solution of *cis*-$[Ru(CO_3)(bpy)_2]$ and (*S*)-phenylalanine was refluxed for 20 h, it was found that phenylalaninato ligands in $[Ru(phe)(bpy)_2]ClO_4\cdot 2H_2O$ isolated had been racemized completely.

9) The k_x' (x; A or B) is determined by

$$k_x' = k_x\cdot[H^+]/K_w$$

The value 5.8×10^{-13} mol^2 dm^{-6} was adopted for ion product of water, K_w, at 100 °C from L. G. Sillén and A. E. Martell, "Stability Constant of Metal-Ion Complexes," Spec. Publ. No. 17, The Chemical Society, London (1964), p. 39.

10) J. L. Bada and R. A. Schroeder, *Naturwissenschaften,* **62**, 71 (1975).

11) J. L. Bada, *Adv. Chem. Ser.,* **106**, 309 (1971).

12) G. G. Smith and R. C. Evans, "Biogeochemistry of Amino Acids," ed by P. E. Hare, T. C. Hoering, and K. King, Wiley Interscience, New York (1980), pp. 257—282.

13) D. A. Buckingham, L. G. Marzilli, and A. M. Sargeson, *J. Am. Chem. Soc.,* **89**, 5133 (1967).

14) A. Pasini and L. Casella, *J. Inorg. Nucl. Chem.,* **36**, 2133 (1974).

15) G. G. Smith, A. Khatib, and G. S. Reddy, *J. Am. Chem. Soc.,* **105**, 293 (1983).

16) T. J. Meyer, *Pure Appl. Chem.,* **58**, 1193 (1986).