5.10 (d, 3.5, OH on C-1), 4.42 (dt, 10.5/3.5, H-1), 4.04 (dd, 10.5/4.0, H-14), 3.10 (m, H-7), 3.07 (m, H-20), 3.04 (s, OMe on C-14), 2.83 (m, 10.7, H-3,16), 2.08 (m, -13.9/10.5/4.3, H-15), 2.01 (m, -13.9/10.5/3.7, H-2), 1.84 (m, -13.9/4.0, H-15), 1.84/1.56 (m, H_2-6,19), 1.81 (m, -13.9/3.5, H-2'), 1.60/1.50 (m, H_2-17), 1.60/1.45 (m, H_2-4), 1.81/1.39 (m, H_2-27,31), 1.52/0.55 (m, H-5,18), 1.25 (m, H_2-29,33), 1.18 (m, H_2-28,32), 0.77/0.76 (t, H_3-30,34).

^{13}C NMR (DMSO-d_6): δ 157.4 (s, C-13), 157.2 (s, C-26), 155.6 (s, C-9), 155.3 (s, C-22), 142.0 (s, C-24), 138.2 (s, C-11), 115.9 (s, C-8), 115.0 (s, C-21), 107.1 (d, C-12), 105.6 (d, C-25), 103.4 (d, C-23), 102.7 (d, C-10), 80.8 (d, C-14), 70.4 (d, C-1), 63.2 (d, C-3), 62.7 (d, C-16), 55.6 (q, OMe on C-14), 47.7 (t, C-2), 45.4 (t, C-15), 40.3 (t, C-4,17), 34.8 (d, C-7), 34.7 (d, C-20), 32.6 (t, C-6,19), 32.4 (t, C-27,31), 30.0/29.9 (t, C-28,32), 26.6 (t, C-5,18), 22.1 (t, C-29,33), 13.9 (q, C-30,34).

Nostocyclophane D. Recrystallized from aqueous EtOH, mp 242-3 °C: $[\alpha]_D$ +10.8° (c 0.4); UV λ_{max} 215 nm (ε 19 600), 228 (13 500), 276 (2080), 283 (2110); CD $[\theta]_{218}$ -28 200, $[\theta]_{230}$ -14 900, $[\theta]_{270}$ -2230, $[\theta]_{280}$ -2600; positive-ion FABMS m/z 653/655/657 (10:6.5:1 MH^+ ion cluster[6] for $C_{36}H_{55}O_6Cl_2$), 637/639/641 (M - Me), 620/622/624 (M - MeOH), 588/590/592 (M - 2 MeOH), 553/555 (M - 2 MeOH - Cl), and 518 (M - 2 MeOH - 2 Cl) at m/z 627, 620, 588, 553, and 518; FDMS m/z 652/654/656 (10:6.5:1 MH^+ ion cluster for $C_{36}H_{54}O_6Cl_2$).

^{1}H NMR (DMSO-d_6): δ (multiplicity, J in Hz, assignment) 8.82/8.80 (s, phenolic OH), 6.15/6.10 (s, H-10,12,23,25), 4.05 (dd, 10.7/3.3, H-1,14), 3.10 (tt, 10.5/3.2, H-7,20), 3.05 (s, OMe on C-1,14), 2.82 (tt, 10.5/3.0, H-3,16), 2.08 (m, -11.3/10.3/3.3, H_a-2,15), 1.85 (m, -11.3/10.7/3.0, H_b-3,15), 1.85/1.58 (m, H_2-6,19), 1.81/1.36 (m, H_2-27,31), 1.62/1.51 (m, H_2-4,17), 1.48/0.54 (m, H_2-5,18), 1.23 (m, H_2-29,33), 1.18 (m, H_2-28,32), 0.77 (t, 7.2, H_3-30,34).

^{13}C NMR (DMSO-d_6): δ 157.5 (s, C-13,26), 155.6 (s, C-9,22), 138.0 (s, C-11,24), 116.1 (s, C-8,21), 107.3 (d, C-12,25), 102.8 (d, C-10,23), 80.9 (d, C-1,14), 62.6 (d, C-3,16), 55.6 (q, OMe on C-1,14), 45.5 (t, C-2,15), 40.3 (t, C-4,17), 34.8 (d, C-7,20), 32.7 (t, C-6,19), 32.5 (t, C-27,31), 30.0 (t, C-28,32), 26.5 (t, C-5,18), 22.1 (t, C-29,33), 13.9 (q, C-30,34).

Uniform Enrichment of Nostocyclophane D. *N. linckia* UTEX B1932 was grown in a 10-L glass bottle on 5.0 g of $NaH^{13}CO_3$ (99 atom %) and 4.0 g of $Na^{15}NO_3$ (99 atom %) as previously described.[8] After 28 days the 8-L culture (alga and medium) was lyophilized. Extraction and workup resulted in the isolation of 2 mg of labeled nostocyclophane D; inspection of its ^{13}C NMR spectrum indicated 37% uniform enrichment.

Acknowledgment. This research was supported by Grant No. CA12623 from the National Cancer Institute, Department of Health and Human Services. A GN500-Omega NMR spectrometer at the University of Hawaii that was used in this study was purchased with a grant from the National Science Foundation. We thank Bradley S. Moore for producing the ^{13}C enriched alga, Faith Caplan and Linda K. Larsen for determining the cytotoxicities, and Drs. Thomas Corbett and Frederick Valeriote (Division of Hematology and Oncology, Wayne State University School of Medicine) for evaluating the compounds for selective cytotoxicity.

Supplementary Material Available: ^{1}H NMR and CD spectra of nostocyclophanes A-D, ^{13}C NMR spectrum of D, and two-dimensional NOESY spectra of A and B (9 pages). Ordering information is given on any current masthead page.

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General Base Catalysis by Hydroxocopper(II) Ion and Existence of Addition Intermediate in Hydrolysis of *m*-(2-Imidazolylazo)phenyl *p*-Toluenesulfonate

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Received January 11, 1991

Rates of the hydrolysis of *m*-(2-imidazolylazo)phenyl p-toluenesulfonate (1) were measured in the presence of Cu(II) ion. Saturation behavior was observed for the dependence on [Cu(II)] of the absorbance (Abs) of 1 or that of the pseudo-first-order rate constant (k_0). The formation constant measured from the dependence on [Cu(II)] of k_0 was much smaller than that of Abs. The binding constant reflected in the Abs data indicates the formation of a 1:1-type complex. The binding constant estimated with the k_0 values may be related to the formation of a 2:1-type complex. This possibility, however, is excluded on the basis of the dependence of the binding constants on pH and the dependence of Abs on [Cu(II)]. Instead, the saturation kinetic behavior agrees with the shift of the rate-determining step between the formation and the breakdown processes of an intermediate upon increase in [Cu(II)]. On the basis of the kinetic data, it is shown that the 1:1-type complex is hydrated to form an addition intermediate, which is subsequently converted into the hydrolysis products, and that hydroxocopper(II) ion participates as a general-base catalyst in the rate-controlling proton-transfer process.

Collection of proofs of existence or nonexistence of intermediates is among the most important tasks in the studies of reaction mechanisms. For nucleophilic substitution on the derivatives of phosphorus oxy acids or sulfur oxy acids, whether the reaction proceeds through the addition-elimination process involving a pentacovalent trigonal-bipyramidal intermediate or through the concerted process involving simultaneous attack of the nucleophile and cleavage of the leaving group has been the center of the mechanistic studies.[1] Results of some recent intensive investigations supported the concerted mechanism in the transfer of phosphoryl, sulfuryl, or sulfonyl groups between various nucleophiles.[2-11] For nucleophilic reactions on aryl

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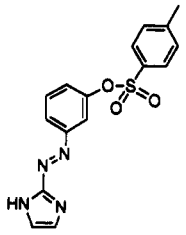
arenesulfonate esters, the concerted mechanism is also supported by several kinetic studies.[12,13] Since the concerted mechanism is favored for the nucleophilic substitution reactions at the phosphorus or sulfur centers by many investigations, demonstration of the existence of an addition intermediate would make a significant contribution to the mechanistic studies in this area.

From kinetic studies on the catalysis by metal ions that act as Lewis acid catalysts in organic reactions, various catalytic roles of the metal ions have been elucidated.[14-16] In particular, we have reported novel catalytic features of metal ions such as the nucleophilic attack by a metal-bound water molecule,[17] participation of a binuclear metal ion as a catalytic unit,[18] and metal ion catalysis by blockade of an inhibitory reverse path.[19]

Although metal-bound hydroxide ions act as nucleophiles in several reactions such as ester hydrolysis, amide hydrolysis, phosphate ester hydrolysis, or alkene hydration,[20-25] general-base assistance by the metal-bound hydroxide ions has not been demonstrated yet. Metal-bound hydroxide ions can be present in neutral or acidic solutions, as well as in the active sites of metalloenzymes. Thus, the general-base catalysis by a metal-bound hydroxide ion may play significant roles in metal ion catalysis.

Roles of metal ions acting as Lewis acids in the nucleophilic reactions on carboxylic or phosphoric acid derivatives have been extensively investigated.[14-24,26,27] However, those on sulfur oxy acids have been rarely studied.[15,28] In this paper, kinetic data measured for the hydrolysis of *m*-(2-imidazolylazo)phenyl *p*-toluenesulfonate (1) in the presence of Cu(II) ion are presented, together with the evidence for the existence of the covalent intermediate and the general-base catalysis by hydroxocopper(II) ion.



1

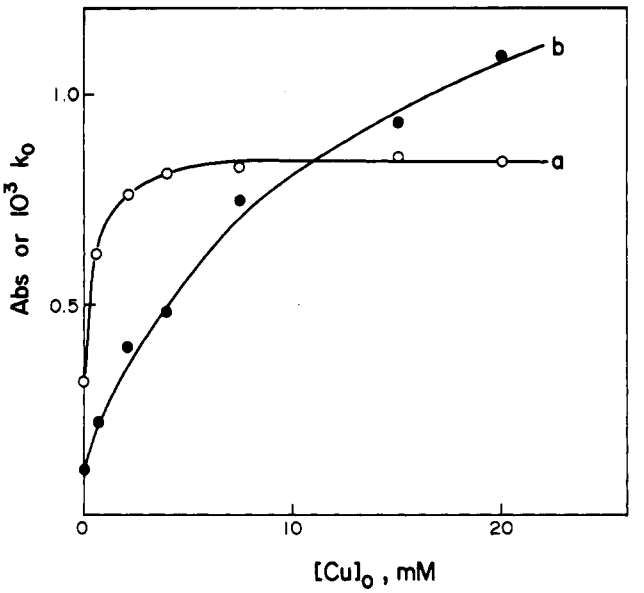


Figure 1. Plot of Abs ($S_0 = 1 \times 10^{-4}$ M) (curve a, ○) and k_0 (curve b, ●) against [Cu(II)] measured for 1 in the presence of Cu(II) ion at pH 3.46.

Experimental Section

Materials. ***m*-(2-Imidazolylazo)phenyl *p*-Toluenesulfonate (1).** Catalytic hydrogenation of *m*-nitrophenyl *p*-toluenesulfonate[29] in methanol with the Pd–C catalyst followed by filtration and evaporation produced *m*-aminophenyl *p*-toluenesulfonate. This (1.1 g), without further purification, was dissolved in dilute HCl (6 mL), and the pH of the solution was adjusted to pH 2. An aqueous solution (5 mL) of $NaNO_2$ (0.36 g) was added to the mixture kept in an ice bath. The resulting mixture was added dropwise to a solution of imidazole (0.44 g) in 6 mL of 3 N NaOH, maintaining the pH of the imidazole solution above pH 10. Thirty minutes after completion of the addition, the reaction mixture was neutralized, and the precipitates formed were separated on a silica gel column eluting with 1:3 (v/v) ethyl acetate–hexane and further purified by recrystallization from ethyl acetate–hexane, mp 190–191 °C. ^1H NMR: δ 2.5 (s, 3 H), 7.0–7.1 (d, 2 H), 7.3–8.0 (m, 8 H). IR (KBr pellet): 1380 cm^{-1} (sulfonate ester). Anal. Calcd for $C_{16}H_{14}N_4O_3S$: C, 56.13; H, 4.12; N, 16.37. Found: C, 56.38; H, 4.03; N, 16.55. Previous studies indicated that the diazotization of imidazole occurs at position 2.[30,31]

***m*-(2-Imidazolylazo)phenol.** Alkaline hydrolysis of 1 in 0.1 M NaOH at 70–80 °C for 2 h and acidification of the mixture at room temperature produced the phenol, which was recrystallized from ethyl acetate–hexane, mp 231–233 °C. Anal. Calcd for $C_9H_8N_4O$: C, 57.44; H, 4.28; N, 29.77. Found: C, 57.11; H, 4.39; N, 29.52.

Cupric chloride was obtained as reported previously.[17] Water was distilled and deionized prior to use in the kinetic studies.

Kinetic Measurements. Reaction rates were measured spectrophotometrically with a Beckman Model DU 64 UV–vis spectrophotometer. Stock solutions of 1 were made in dimethyl sulfoxide. The reaction mixtures for the kinetic measurements contained 1% (v/v) dimethyl sulfoxide. The initially added concentration (S_0) of the substrates was 1×10^{-4} M. The dependence of absorbance of 1 on [Cu(II)] at various pHs was measured immediately after mixing 1 with Cu(II). The kinetics of the hydrolysis of 1 in the absence and presence of Cu(II) ion were measured at 450 and 430 nm, respectively. Rates were measured at 25 °C and an ionic strength of 0.5 M (adjusted with NaCl) in the presence of 0.01 M monochloroacetate (pH 2.2–3.5) or acetate (pH 3.6–4.3) buffer, unless noted otherwise. Temperature was controlled to within ±0.1 °C with a Haake E12 circulator. Pseudo-first-order kinetics were observed up to at least

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Table I. Values of K_{f1}^{app}, K_{f2}^{app}, and k_c^{app} Measured for the Cu(II)-Catalyzed Hydrolysis of 1[a]

pH	K_{f1}^{app} (M^{-1})	K_{f2}^{app} (M^{-1})	k_c^{app} (10^{-3} s^{-1})
2.70	1060 ± 40		
2.95	1430 ± 10		
3.20	1700 ± 40		
3.46	1990 ± 20	95.2 ± 22.0	1.55 ± 0.14
3.72	2730 ± 460	248 ± 30	1.97 ± 0.07
4.00	4460 ± 540	490 ± 69	1.92 ± 0.08
4.25	5090 ± 300	555 ± 21	2.08 ± 0.02

[a] The estimated values of K_{f1}^{app} are the same as those of K_f^{Abs}, those of K_{f2}^{app} are the same as those of K_f^{kin}, and those of k_c^{app} are the same as those of k_{cat} or k_{c2}^{app} (see text).

3 half-lives. The UV–vis spectra of the product solutions obtained in the kinetic studies agreed with those prepared with the isolated hydrolysis products. Kinetic studies in the presence of Cu(II) ion were not extended beyond pH 4.3 due to the limited solubility of the metal ion at higher pHs.

Results

Addition of Cu(II) ion to the solution of 1 results in changes in the absorbance (Abs) of the substrate (S) as well as the pseudo-first-order rate constant (k_0) for the hydrolysis of the substrate, as illustrated in Figure 1. The saturation behavior observed for the dependence of Abs on [Cu(II)][32] indicates formation of a complex (Cu(II)S) between the substrate and metal ion. Thus, the dependence of Abs on [Cu(II)] is described by Scheme I and eq 1, in which Abs_S and Abs_{MS} represent the absorbance observed when the substrate is present exclusively as S and Cu(II)S, respectively.

Scheme I

$$S \underset{}{\overset{K_f^{Abs}[Cu(II)]}{\rightleftharpoons}} Cu(II)S$$

$$Abs = (Abs_S + Abs_{MS}K_f^{Abs}[Cu(II)])/(1 + K_f^{Abs}[Cu(II)]) \quad (1)$$

Saturation kinetic behavior observed in the presence of a catalyst is most often analyzed by assuming the formation of a complex between the substrate and the catalyst. A typical example is the analysis of kinetic data for enzymatic reactions in terms of the Michaelis–Menten scheme. Similarly, the dependence of k_0 on [Cu(II)] at a constant pH is analyzed in terms of Scheme II and eq 2.

$$k_0 = (k_{sp} + k_{cat}K_f^{kin}[Cu(II)])/(1 + K_f^{kin}[Cu(II)]) \quad (2)$$

The formation constant (K_f^{Abs}) revealed by the dependence of Abs on [Cu(II)] is much greater than that (K_f^{kin}) by the dependence of k_0 on [Cu(II)].[33] At pH 3.46, for example, binding of the substrate to the Cu(II) ion is almost complete at 2 mM Cu(II) as judged by the dependence of Abs on [Cu(II)] (Figure 1; curve a), but k_0 manifests saturation behavior at much greater [Cu(II)] concentrations (Figure 1; curve b). Here, K_f^{Abs} is 20 times greater than K_f^{kin}. The disagreement between K_f^{Abs} and K_f^{kin} indicates that Scheme II is not appropriate for the description of the kinetic behavior.

According to the Abs data, formation of the 1:1-type complex from Cu(II) and 1 must be complete when [Cu(II)] ≫ $1/K_f^{Abs}$. Thus, the kinetic data measured over this range of [Cu(II)] represent the reaction of Cu(II)S in the presence of Cu(II) ion. If the saturation kinetic data observed for 1 are to be analyzed in terms of the formation of a complex between Cu(II)S and Cu(II) ion, a 2:1-type complex ($Cu(II)_2S$) is to be involved. Then, the reaction of 1 would be described as Scheme III, for which expressions of Abs and k_0 are derived as eqs 3–5.[34] When [Cu(II)] ≪ $1/K_{f2}^{app}$,

$$Abs = (Abs_S + Abs_{MS}K_{f1}^{app}[Cu(II)])/(1 + K_{f1}^{app}[Cu(II)]) \quad (3)$$

When [Cu(II)] ≫ $1/K_{f1}^{app}$,

$$Abs = (Abs_{MS} + K_{f2}^{app}Abs_{MMS}[Cu(II)])/(1 + K_{f2}^{app}[Cu(II)]) \quad (4)$$

$$k_0 = (k_{c1}^{app} + k_{c2}^{app}K_{f2}^{app}[Cu(II)])/(1 + K_{f2}^{app}[Cu(II)]) \quad (5)[35]$$

Here, Abs_{MMS} is the Abs expected when the substrate is present exclusively as $Cu(II)_2S$.

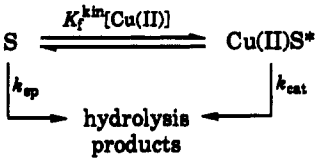
Alternatively, the saturation kinetic behavior observed in the presence of a catalyst can be explained by assuming that the rate-determining step changes as the concentration of the catalyst is raised, as indicated in Scheme IV (rate expressions, eqs 6–8) or V (rate expressions, eqs 9 and 10).[36] When [Cu(II)] ≫ $1/K_{f1}^{app}$ and $k_0 > k_{sp}$,

$$k_0 = k_c^{app}K_{f2}^{app}[Cu(II)]/(1 + K_{f2}^{app}[Cu(II)]) \quad (6)$$

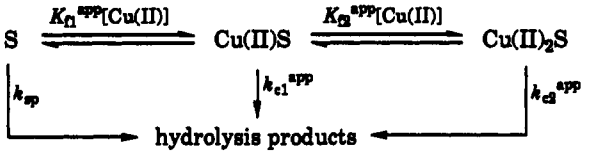
$$K_{f2}^{app} = k_{-2}/k_3 \quad (7)$$

$$k_c^{app} = k_2k_3/k_{-2} \quad (8)$$

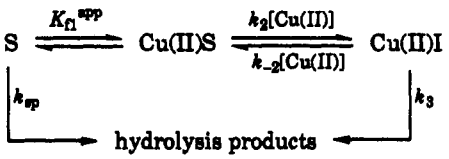
Scheme II



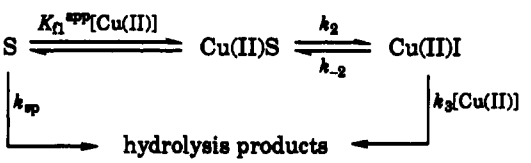
Scheme III



Scheme IV



Scheme V



(32) When the initially added concentration of the substrate was not negligible compared with that of Cu(II) ion ($[Cu(II)]_0$), [Cu(II)] was calculated by correcting $[Cu(II)]_0$ with the estimated formation constant for the metal–substrate complex.

(33) Dependence of Abs or k_0 on [Cu(II)] or the pH dependence of K_f values was analyzed by a computer program based on the nonlinear regression method reported in the literature: Yamaoka, K.;Tanigawara, Y., Nakagawa, T.; Uno, T. *J. Pharm. Dyn.* **1981**, *4*, 879.

(34) The general expression for Abs is $Abs = (Abs_S + K_{f1}^{app}Abs_{MS}[Cu(II)] + K_{f1}^{app}K_{f2}^{app}Abs_{MMS}[Cu(II)]^2)/(1 + K_{f1}^{app}[Cu(II)] + K_{f1}^{app}K_{f2}^{app}[Cu(II)]^2)$. For derivation of eqs 3 and 4, it is considered that Abs_{MMS} is similar to Abs_{MS} and that Abs_{MS} is greater than Abs_S (Figure 1).

(35) For derivation of eq 5, it is taken into consideration that $k_0 > k_{sp}$.

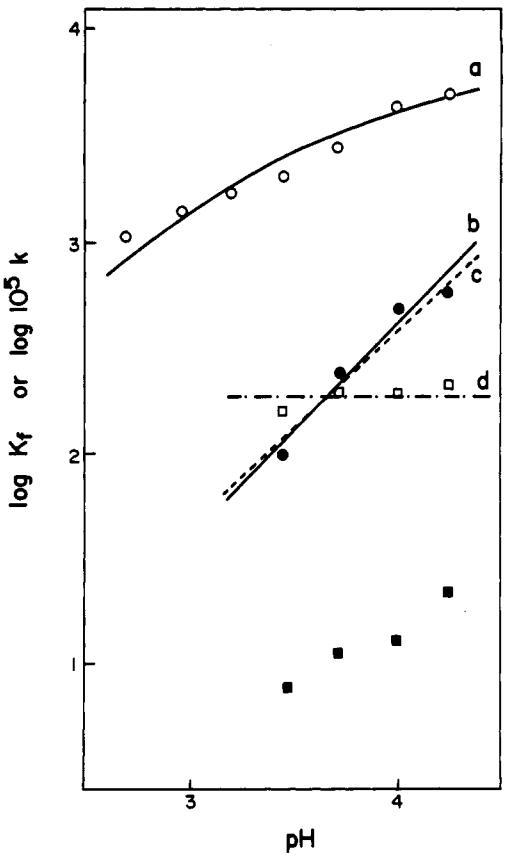


Figure 2. pH profiles of log K_{f1}^{app} (○), log K_{f2}^{app} (●), log $10^5 k_c^{app}$ (□), and log $10^5 k_{sp}$ (■). Curves a and c are constructed according to eqs 11 and 12, respectively, on the basis of the parameter values summarized in Table II. Theoretical curve for log K_{f1}^{app} built with eq 13 is almost identical with curve a. Linear line b is drawn with slope of 1.0 and linear line d with slope of 0. The values of k_{sp} measured in the presence of 0.01 or 0.03 M buffer indicate that the general-acid–base catalysis by the buffer may be responsible for up to 20% of the k_{sp} values (■) measured in the presence of 0.01 M buffer.

When [Cu(II)] ≫ $1/K_{f1}^{app}$ and $k_0 > k_{sp}$, k_0 is also expressed as eq 6, but K_{f2}^{app} and k_c^{app} are given as

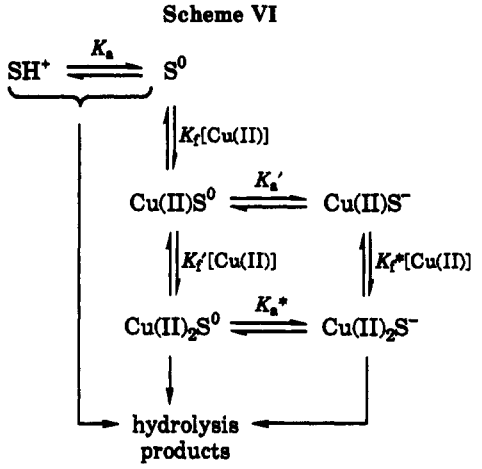
$$K_{f2}^{app} = k_3/k_{-2} \quad (9)$$

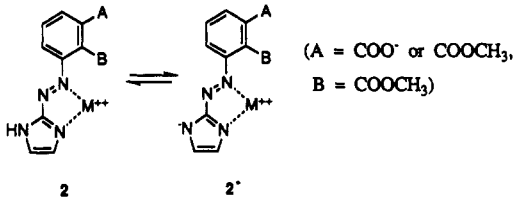
$$k_c^{app} = k_2 \quad (10)$$

Analysis of Abs data in terms of Scheme III produced K_{f1}^{app} values that were the same as K_f^{Abs} values obtained on the basis of Scheme I. Analysis of the rate data in terms of Scheme IV or V led to the values of K_{f2}^{app} and k_c^{app} that were identical with K_{f2}^{app} and k_{c2}^{app}, respectively, estimated by Scheme III, or with K_f^{kin} and k_{cat}, respectively, analyzed by Scheme II.[37] The values of K_{f1}^{app}, K_{f2}^{app}, and k_c^{app} are summarized in Table I and Figure 2. The proportionality between K_{f2}^{app} and [OH^-] and the pH independence of k_c^{app} are seen in Figure 2.

Compound 1 contains an imidazole ring that can be protonated over the pH range examined. The spectral titration (not shown) of 1 at 430 nm indicated a pK_a value of 3.28 for the conjugate acid of 1. In the 1:1- or 2:1-type Cu(II) complex of 1, the imidazole NH group may be deprotonated over the pH range studied. For example, the pKs of the imidazole NH group 2 in the Cu(II) or Ni(II) complexes of 2-imidazoleazo compounds similar to 1 were estimated as 3.5–5.7 in a previous study.[30] Analysis of the pH dependence of Abs_{MS} at pH 2.7–4.3, however, did not indicate ionization of the imidazole NH of Cu(II)1 over this pH range.

Scheme VI





2 2⁻

Spectral titration (not shown) of *m*-(2-imidazolylazo)-phenol revealed pK = 8.76 for the free phenol and pK = 7.47 for the Ni(II)-complexed phenol. The spectral titration of the Cu(II)-complexed phenol was not performed due to the low solubility of Cu(II) ion at pH > 4.3. Since Cu(II) ion is a stronger Lewis acid than Ni(II) ion (Irving–Williams order),[38-40] the pK of the Cu(II)-complexed phenol should be smaller than that of the Ni(II)-complexed one and is estimated as 5–7.[41]

Discussion

pH Dependence of Binding Constants. Hydrolysis of 1 is accelerated by Cu(II), although the degree of acceleration is not very large. Much more remarkable to note than the degree of acceleration are the participation of two Cu(II) ions in the catalysis and the large apparent binding constant for the second Cu(II) ion.

The saturation behavior observed for k_0 for 1 can be explained in terms of Scheme III, IV, or V. The formation

(36) Hydrolysis of Cu(II)S may be included in Schemes IV and V. But, its contribution to the overall rate is not significant, and addition of this path to the reaction schemes does not affect the estimated values of K_{f2}^{app} and k_c^{app} appreciably.

(37) As illustrated in Figure 1, the hydrolysis of 1 in the presence of Cu(II) ion is dominated by the reaction of Cu(II)$_2$S, and the reactivity (k_{c1}^{app}) of Cu(II)S is difficult to estimate. When $k_{c1}^{app} = k_{sp}$, eq 5 is identical with eq 2. The values of K_{f2}^{app} and k_{c2}^{app} are estimated by assuming that $k_{c1}^{app} = k_{sp}$ are not considerably different from those estimated by neglecting k_{c1}^{app}. The weighted averages of K_{f2}^{app} and k_{c2}^{app} values estimated by the two methods are presented in Table I and Figure 2.

(38) Basolo, F.; Pearson, R. G. *Mechanisms of Inorganic Reactions*, 2nd ed.; Wiley: New York, 1967; pp 32 and 80.

(39) Cotton, F. A.; Wilkins, G. *Advanced Inorganic Chemistry*, 3rd ed.; Interscience: New York, 1972; p 596.

(40) Irving, H.; Williams, R. J. P. *J. Chem. Soc.* **1953**, 3192.

(41) The pK of the oxime group in 2-pyridinecarboxaldoxime is 10.0, whereas it is 6.3 and 3.2, respectively, in the Ni(II) and Cu(II) complexes of 2-pyridinecarboxaldoxime: Suh, J.; Lee, J. D. *Inorg. Chem.* **1985**, *24*, 3088. Suh, J.; Chang, B. *Bioorg. Chem.* **1987**, *15*, 167. Similarly, the pK of the Cu(II) complex of *m*-(2-imidazolylazo)phenol would be appreciably smaller than that of the corresponding Ni(II) complex. Spectral titration of the Cu(II) complex of the phenol at below pH 4.3 did not indicate ionization of the phenol. Thus, the lower limit of the pK of the Cu(II) complex of *m*-(2-imidazolylazo)phenol is set as 5.

Table II. Values of Parameters Estimated from the Analysis of the pH Profiles of K_{f1}^{app} and K_{f2}^{app} According to Scheme VI

parameter	value
pK_a'	4.69 ± 0.26^a
pK_a^*	$0.69^{b,c}$
K_f (M^{-1})	$4000 \pm 210^{a,d}$
K_f' (M^{-1})	0.22^b

[a] Analyzed according to eq 11. [b] Analyzed according to eq 12 by using the best value of pK_a' (4.69) estimated by analysis of the pH profile of K_{f1}^{app}. [c] Regardless of the assumed value (0–14) of pK_a', the value of pK_a^* estimated according to eq 12 does not exceed 1.0. [d] When analyzed in terms of eq 13 neglecting the ionization process of pK_a', K_f is estimated as 4440 ± 200 M^{-1}.

of a stable 2:1-type complex ($Cu(II)_2S$) is assumed in Scheme III. Analysis of the kinetic data in terms of this scheme leads to the formation constant ($K_{f1}^{app}K_{f2}^{app}$) for $Cu(II)_2S$ of $(2–30) \times 10^5$ M^{-2} at pH 3.5–4.3, with the substrate being almost completely converted into $Cu(II)_2S$ at 0.02–0.1 M [Cu(II)].

In order to analyze the pH dependencies of various kinetic parameters illustrated in Figure 2, Scheme III is to be revised as Scheme VI. Since the azo nitrogen atom and the imidazole nitrogen atom are the chelating sites of 1,[30,31] protonation of the imidazole portion would inhibit complexation of 1 to Cu(II) ion.

For Scheme VI, K_{f1}^{app} and K_{f2}^{app} of eqs 3–5 are expressed by eqs 11 and 12, respectively. Analysis of the pH profile of K_{f1}^{app} according to eq 11 by using the value of $pK_a = 3.28$ obtained from the spectral titration of 1 led to the values of K_f and pK_a'. From the analysis of the pH profiles of K_{f2}^{app} according to eq 12, K_f' and pK_a^* were estimated. The parameter values thus obtained are summarized in Table II. Without invoking the ionization step of K_a^* in Scheme VI, the shape of the pH profile of K_{f2}^{app} cannot be explained.

$$K_{f1}^{app} = K_f(1 + K_a'/[H^+])/(1 + [H^+]/K_a) \quad (11)$$

$$K_{f2}^{app} = K_f'(1 + K_a^*/[H^+])/(1 + K_a'/[H^+]) \quad (12)$$

For the mechanism of Schemes III and VI, the substrate is present as the mixture of the 1:1-type and the 2:1-type complexes when $[Cu(II)] \gg 1/K_{f1}^{app}$, with the 2:1-type complex being predominant at larger [Cu(II)] concentrations. The dependence of Abs on [Cu(II)] for 1 measured at several wavelengths and pH 2.7–4.3 (e.g., line a of Figure 1), however, indicates that Abs does not change when [Cu(II)] is raised once the fraction of the uncomplexes substrate becomes negligible ($[Cu(II)] \gg 1/K_{f1}^{app}$). This can be explained only when the spectra of the 1:1-type complex and the 2:1-type complex are exactly the same over the pH range examined. Both the spectral titration of the Cu(II) complex of 1 and the analysis of pH dependence of K_{f1}^{app} and K_{f2}^{app} indicate that the 1:1-type complex does not ionize and the 2:1-type complex ionizes completely over the pH range investigated in the present study if the mechanism of Schemes III and VI is operative. Since the deprotonation of the imidazole NH group of an imidazolylazo metal complex changes the visible spectrum of the complex remarkably,[30,31] the spectra measured at pH 2.7–4.3 for the 1:1-type complex and the 2:1-type complex should differ considerably from each other in marked contradiction to the Abs data. The mechanism of Schemes III and IV, which assumes formation of a stable $Cu(II)_2S$ complex, is therefore incompatible with the results.

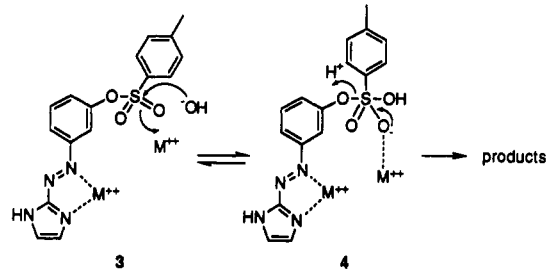
In the case of Scheme IV or V, the steady-state concentration of the intermediate (Cu(II)I) would be negligible compared with Cu(II)S if it is an unstable intermediate. Thus, Abs of 1 would not be affected by further increase in [Cu(II)], once 1 is fully converted into Cu(II)S, in agreement with the data obtained for the dependence of Abs on [Cu(II)]. The pH profiles of K_{f2}^{app} and k_c^{app} are readily explained in terms of Schemes IV and V. The proportionality (Figure 2) between K_{f2}^{app} and $[OH^-]$ is accommodated by assuming that k_{-2}/k_3 (for Scheme IV) or k_3/k_{-2} (for Scheme V) is proportional to $[OH^-]$, and the pH independence of k_c^{app} is accounted for by assuming that k_2k_3/k_{-2} (for Scheme IV) or k_2 (for Scheme V) is independent of pH.

When Schemes I, IV, and V are modified to include the ionization (K_a) of 1, the expression of K_f^{Abs} and, therefore, K_{f1}^{app} is derived as eq 13.

$$K_f^{Abs} = K_{f1}^{app} = K_f/(1 + [H^+]/K_a) \quad (13)$$

Here, K_f is the constant for the binding of the neutral form of 1 to Cu(II).

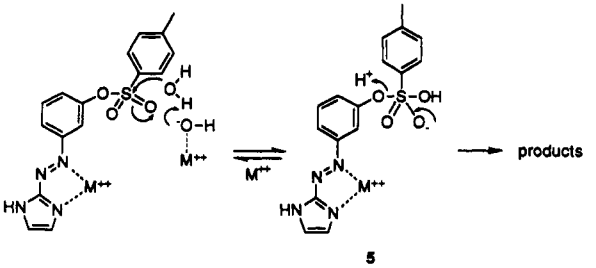
Mechanism of Catalysis. If the mechanism of Scheme IV is operative, the pH profiles of K_{f2}^{app} and k_c^{app} require that k_{-2}/k_3 be proportional to $[OH^-]$ and k_2k_3/k_{-2} be independent of pH. In the mechanism of 3–4, k_2 and k_3 are



3 4

proportional to $[OH^-]$ and $[H^+]$, respectively, and k_{-2} is independent of pH, in agreement with the kinetic data. According to this mechanism, however, expulsion of the phenolate anion from 4 (k_3 step) requires assistance from H^+, whereas that of much more basic OH^- ion (k_{-2} step) does not. This mechanism is, therefore, unlikely.

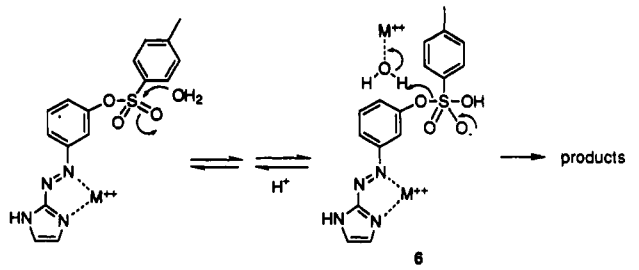
The mechanism of 5 may be considered as an alternative for Scheme IV. The pK of the Cu(II)-bound water molecule of aquocopper(II) ion is 6.8,[38] and $[Cu(II)OH^-]$ is proportional to $[Cu(II)][OH^-]$ at pH < 4.3. This mechanism appears, however, to be unreasonable since expulsion of the phenolate anion from 5 (k_3 step) requires assistance from H^+ whereas that of much more basic OH^- from 5 (k_{-2} step) occurs only with assistance from very weakly acidic aquocopper(II) ion.



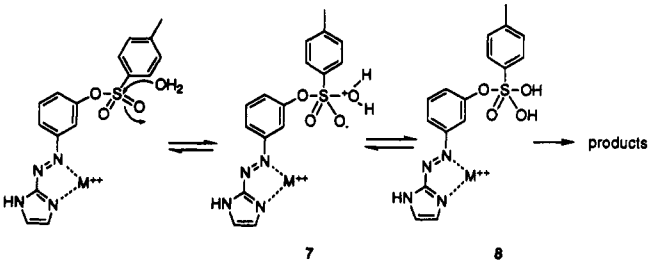
5

Analysis of the kinetic data in terms of Scheme V requires that k_2 be independent of pH and k_3/k_{-2} be proportional to $[OH^-]$ at pH 3.4–4.3. In the mechanism of 6, k_2 and k_3 are independent of pH and k_{-2} is proportional to $[H^+]$, in agreement with the kinetic data. This mechanism is also unlikely, as the expulsion of the phenolate is assisted by a general acid that is not more acidic (pK

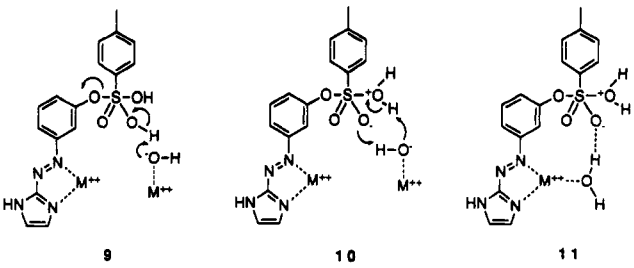
of Cu(II)OH_2, 6.8) than its conjugate acid (pK of the phenol, 5–7).[42,43]



Alternatively, the mechanism of 7–8 can be proposed for Scheme V. If either 7 or 8 corresponds to Cu(II)I and the breakdown of Cu(II)I is subject to catalysis by Cu(II) and OH^-, the observed kinetic data are accounted for with k_2 and k_{-2} being independent of pH and k_3 proportional to [OH^-]. If 8 is taken as Cu(II)I, the hydroxocopper(II) ion acts as a general-base abstracting proton from the sulfur-linked hydroxyl group 9. This mechanism, however, appears to be unlikely[43,44] since at low [Cu(II)] the k_{-2} step is faster than the k_3 step and, thus, expulsion of OH^- is easier than the phenolate anion from 8.



Although several mechanisms that conform to the kinetic data are considered, all except for one mechanism are excluded. In the only unexcluded mechanism, 7 represents Cu(II)I and hydroxocopper(II) ion participates as general-base catalyst for the proton-transfer process. A possible process involved in the general-base catalysis is illustrated in 10.[45,46] There are several precedents in which addition intermediates revert to reactants very fast and the intermediates are trapped by rate-controlling proton transfer catalyzed by general acids or bases.[47,48]



In summary, the Cu(II) complex of 1 is hydrated to form an addition intermediate 7, which is subsequently converted into 8 by proton transfer, in the mechanism most consistent with the data. Furthermore, the proton-transfer process is catalyzed by hydroxocopper(II) ion and the rate-controlling step is shifted from the proton-transfer process to the formation of the addition intermediate when the proton transfer is accelerated sufficiently.

Novel Mechanistic Features. Existence of a covalent intermediate can be demonstrated by several methods.[49,50] If the intermediate is very stable, it may be isolated and its structure can be characterized. In most cases, however, the intermediates are very unstable and their existence is proved rather indirectly. In the present study, evidence supporting existence of the intermediate is obtained by changing the rate-controlling step from the breakdown of the intermediate to the formation of the intermediate through raising the catalyst concentration. This could be considered as the first case in which the existence of an intermediate is demonstrated in the hydrolysis of an aryl sulfonate ester.

For the hydrolysis of aryl arenesulfonate esters, a wide spectrum of mechanisms is available with regard to the timing of the attack by the nucleophile and the cleavage of the leaving group. The actual reaction path would depend on the structure of the substrate in addition to several other factors. Thus, the existence of a covalent intermediate for the hydrolysis of 1 in the presence of Cu(II) does not necessarily indicate the general applicability of the addition–elimination mechanism for the sulfonate ester hydrolysis. It is possible that the negative charge developed on the oxygen atom of addition intermediate 7 may interact with the metal-bound water through hydrogen bonding 10,[51] leading to stabilization of the intermediate. This may affect the reaction path to tilt toward the addition–elimination mechanism. Moreover, this may partly explain why no evidence has been obtained for the existence of addition intermediates in the hydrolysis of simpler sulfonate esters in the absence of transition metal ions.

Ionization of water molecule is enhanced upon coordination to metal ions, and hydroxometal ions can be present in high concentrations even in weakly acidic aqueous solutions.[38] The catalytic action of metal-bound hydroxide ions has been observed in many organic reactions catalyzed by metal ions.[20–25] In all of the reported reactions, the metal-bound hydroxide ions act as nucleophiles. Metal-

(42) Jencks, W. P. *Chem. Rev.* **1972**, *72*, 705; *J. Am. Chem. Soc.* **1972**, *94*, 4731.

(43) For the mechanism of 6, the hydration of Cu(II)I might proceed through the protonation of the sulfonyl oxygen and the subsequent attack by OH^- at the sulfonyl sulfur. For the mechanism of 7–8, the same processes would form 8 directly from Cu(II)I. These alternative mechanisms, however, are excluded by the same arguments made against the assignment of 6 or 8 as Cu(II)I.

(44) In order to observe general-base assistance by hydroxocopper(II) ion in the breakdown of 8, the pK of the hydroxyl group connected to the sulfur atom in 8 should be considerably greater than 7. In this regard, it is noteworthy that the pK of benzenesulfinic acid ($C_6H_5S(O)OH$) is 2.0: Jencks, W. P.; Regenstein, J. In *Handbook of Biochemistry and Molecular Biology*, 3rd ed.; Fasman G. D., Ed.; CRC Press: Cleveland, 1976; Vol I, p 317. However, it is not easy to predict the acidity of the oxyacids of pentacovalent sulfur since the apical S–O bond lengths in the pentacoordinate species are substantially increased over the lengths in the tetrahedral structures (Perkins, C. W.; Wilson, S. R.; Martin, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 3209 and references therein) and, thus, the atoms occupying the apical positions might bear extra negative charges.[1]

(45) If the hydroxyl group linked to the sulfur atom is sufficiently acidic, the anion of 8 instead of 8 itself would be formed as an intermediate. Then, hydroxocopper(II) ion would catalyze the deprotonation of 7 to form this intermediate.

(46) It is possible that the preassociation catalysis or spectator catalysis (Schowen, R. L. In *Mechanistic Principles of Enzyme Activity*; Liebman, J. F., Greenberg, A., Eds.; VCH: New York, 1988; Chapter 4) is involved in the action of the hydroxocopper(II) ion.

(47) Jencks, W. P. *Acc. Chem. Res.* **1976**, *12*, 425.

(48) Other general bases may also catalyze the proton-transfer process. However, effects of other general bases were not studied in the presence of Cu(II) ion as they would not only affect the proton transfer process but also modify the reactivity of Cu(II) ion by coordinating to the metal ion.

(49) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; pp 44–66.

(50) Jones, R. A. Y. *Physical and Mechanistic Organic Chemistry*, 2nd ed.; Cambridge University Press: Cambridge, 1984; pp 5–9.

(51) Although a 10-membered ring is proposed in 11, rotation around only four single bonds is free, affecting the conformation of the ring.

bound hydroxide ions can also act as general bases, but this possibility has not been experimentally demonstrated yet. The present study of the hydrolysis of 1 in the presence of Cu(II) ion is the first evidence obtained for the general base catalysis by metal-bound hydroxide ions.

In many metalloenzymes, metal ions act as Lewis acids.[52] In the action of such a metalloenzyme, the metal-bound water molecule and the metal-bound hydroxide ion as well as the metal ion itself can act as catalytic functional groups. The nucleophilic attack by metal-bound water[17] or hydroxide ion[20-25] has been demonstrated in model systems. The general-base action of metal-bound hydroxide ion demonstrated in the present study expands the repertoire of the catalytic roles of metal ions in metalloenzymes.

(52) Vallee, B. L.; Wacker, W. E. C. *Handbook of Biochemistry and Molecular Biology*; 3rd ed.; Fasman, G. D., Ed.; CRC Press: Cleveland, 1976; Vol. II, pp 276-292.

Acknowledgment. This work was supported by Organic Chemistry Research Center and Korea Science and Engineering Foundation.

A Highly Stereoselective Synthesis of (*E*)-Alkene Dipeptide Isosteres via Organocyanocopper–Lewis Acid Mediated Reaction

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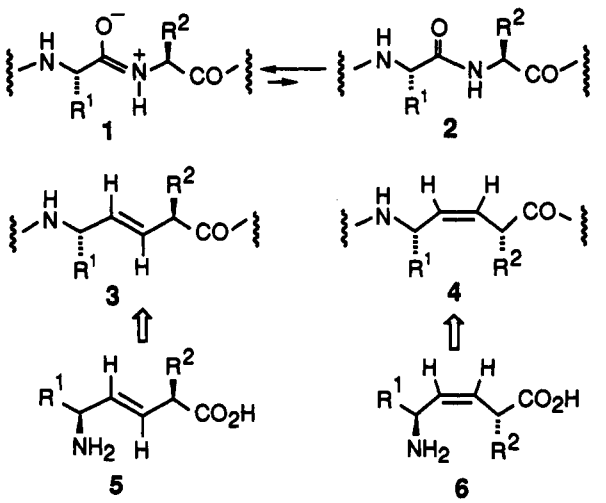
Received January 3, 1991

A stereoselective synthesis of protected (*E*)-alkene dipeptide isosteres by the reaction of the mesylates of homochiral δ-aminated γ-hydroxy (*E*)-α,β-enoates with either RCu(CN)Li·BF_3 or RCu(CN)MgX·BF_3 reagent is described. The degree of diastereoselectivity has been found to be uniformly high except for the serine- and threonine-derived acetonides **77** and **81**. The synthesis permits the introduction of sterically hindered appendages such as isopropyl and *tert*-butyl groups at the α position to the ester group. This methodology provides a new route to a wide range of modified (*E*)-alkene peptide mimics that may have biological importance.

In recent years, increasing interest has been shown in the backbone modification of amide bonds in biologically active peptides.[1] The major purpose in this area deals with stabilizing a given peptide toward enzymatic degradation by in vivo proteases or imparting enzyme inhibitory activity to the synthesized peptide mimic.[2] The peptide bond in polypeptides and proteins generally assumes the trans amide bond configuration **1**, since its cis counterpart induces unfavorable steric interactions.[3a-c] In fexible peptides, a proline-generated cis configurational isomer generally accounts for up to 30% of the total cis/trans population.[3d] Consequently, free rotation around the CO–NH bond axis is retarded (Scheme I).[3]

The (*E*)-CH=CH bonding in a peptide mimic (**3**) closely resembles the three-dimensional structure (bond length, bond angle, and rigidity) of the parent amide (**1** and **2**).[2,3] Thus, replacement of an amide bond by a (*E*)-CH=CH bond should not significantly alter the overall conformation

Scheme I



of a peptide molecule, and, hence, its biological activity, provided that the replaced amide bond is not directly involved in either the secondary or tertiary structure of the peptide or the mechanism whereby the biological response is elicited.[2] It has recently been shown that peptide analogues **4** having a (*Z*)-alkene dipeptide isostere (**6**) were considerably less bioactive than peptide mimics **3** involving an (*E*)-alkene isostere (**5**).[4] The interest in these (*E*)-

(1) (a) Spatola, A. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Marcel Dekker: New York, 1983; Vol. 7, p 267. (b) According to IUPAC rules, the structure inside the bracket following ψ is the unit substituting for the amide bond. For nomenclature, see: IUPAC-IUB Joint Commission on Biochemical Nomenclature, *Eur. J. Biochem.* **1984**, *138*, 9.

(2) Hann, M. M.; Sammes, P. G.; Kennewell, P. D.; Taylor, J. B. *J. Chem. Soc., Chem. Commun.* **1980**, 234. Hann, M. M.; Sammes, P. G.; Kennewell, P. D.; Taylor, J. B. *J. Chem. Soc., Perkin Trans. I* **1982**, 307.

(3) (a) Dickerson, R. E.; Geis, I. In *The Structure and Action of Proteins*; Harper & Row: New York, Evanston, London, 1969; p 13. (b) Schulz, G. E.; Schirmer, R. H. In *Principles of Protein Structure*; Springer-Verlag: New York, Heidelberg, Berlin, 1979; p 18. (c) Sukumaran, D. K.; Prorok, M.; Lawrence, D. S. *J. Am. Chem. Soc.* **1991**, *113*, 706 and references cited. (d) London, R. E.; Matwiyoff, N. A.; Stewart, J. M.; Cann, J. R. *Biochemistry* **1978**, *17*, 2277.

(4) Kaltenbronn, J. S.; Hudspeth, J. P.; Lunney, E. A.; Michniewicz, B. M.; Nicolaides, E. D.; Repine, J. T.; Roark, W. H.; Stier, M. A.; Tinney, F. J.; Woo, P. K. W.; Essenberg, A. D. *J. Med. Chem.* **1990**, *33*, 838.

0022-3263/91/1956-4370$02.50/0