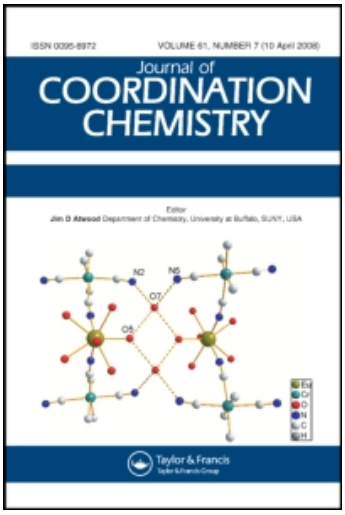

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Free Metal Ion Depletion by Good's Buffers. IV. Bicine 1:1 and 2:1 Complexes with Mg(II), Ca(II), Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)

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FREE METAL ION DEPLETION BY GOOD'S BUFFERS. IV. BICINE 1:1 AND 2:1 COMPLEXES WITH Mg(II), Ca(II), Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)

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The interaction of "Good's" buffers with a variety of metal ions has been assessed.

Keywords: Good's buffers, bicine, complexes, stability constants, biological systems

INTRODUCTION

"Good's" buffers[1] are presently used as a matter of routine in medical and biochemical studies under the assumption that they undergo little if any interaction with biologically important metal ions. Previously, it was shown[2] that the widespread use of "Good's" buffers could be, in part, responsible for the many conflicting data and conclusions derived by investigators studying identical metal cation protein systems at the same pH in carefully executed experiments. We have shown[2] that not only do "Good's" compounds[1] buffer H^+ concentrations as well as metal ion concentrations but also the resultant metal complexes buffer H^+ and metal ion concentrations. In a continuing study,[3] we report potentiometric, spectrophotometric, and nuclear magnetic resonance studies on the metal chelates of the "Good's" buffer, bicine, *N*,*N*-bis(2-hydroxyethyl)glycine.

EXPERIMENTAL

Reagents

Bicine was purchased from Boehringer-Mannheim Biochemicals and met standards of $\geq 99\%$ purity based on potentiometric titrations. Baker analyzed $Cu(NO_3)_2 \cdot 3H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, $Co(NO_3)_2 \cdot 6H_2O$, and Fisher certified 50% aqueous $Mn(NO_3)_2$ were used in the preparation of all metal ion solutions, which were standardized *via* ion-exchange techniques. Aliquots of the solutions were passed through Dowex 50W-X8 cation-exchange resin, and the resultant solutions were titrated with standardized NaOH or $N(CH_3)_4OH$ solutions.

Baker analyzed $Mg(NO_3)_2 \cdot 6H_2O$, $Ca(NO_3)_2 \cdot 4H_2O$, and $Zn(NO_3)_2 \cdot 6H_2O$ were reacted with 2,2-dimethoxypropane (DMP) under gentle reflux for 1 hour. The resultant solids (DMP reacts with H_2O to form methanol and acetone) were filtered,

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dried at 80°C under vacuum, and dissolved in Gold Label Aldrich 99.8% D D_2O. The metal ion solutions (1.8–2.5 M) were then standardized *via* ion-exchange techniques described above. Sodium deuteroxide solutions were prepared by dissolving freshly cut metallic sodium in 99.8% D D_2O and standardized with KHP using a phenolphthalein endpoint. These solutions were used in the NMR studies to reduce the size of the HDO peak.

Potentiometric Measurements

A Corning Digital 130 research model pH meter was used to determine hydrogen ion concentration in all potentiometric studies, which were carried out in a double walled cell of 50 cm^3 capacity. The titration cell was fitted with Fisher glass and calomel extension electrodes, a microburette delivery tube, and a nitrogen inlet tube. The temperature of all solutions was maintained at 25.00 ± .05°C by circulation of thermostatted water through the outer jacket of the cell, and the solutions were stirred by a magnetic stirrer. Ionic strengths of all solutions (~0.008 M M^{2+}) were maintained at 0.10, (KNO_3 or $N(CH_3)_4NO_3$) by the addition of any appropriate amount from 1.0 M stock solutions. All titrations were performed in triplicate.

The glass electrode was calibrated in terms of $-\log[H^+]$ according to the procedure of Rajan and Martell[4] using HCl and NaOH solutions; therefore the pH meter read $[H^+]$ and not a_{H^+}. Equilibrium constants were determined on the IBM 360–65 digital computer using Bjerrum's[5] method.

Visible Spectra

Spectra of 1:1 and 2:1 bicine to M^{2+} ($2.0–5.0 \times 10^{-3}$ M) solutions from **a** = 0.0 to **a** = 2.0 (3.0), moles of base per mole of ligand, were obtained on a Cary 14R recording spectrophotometer at ambient temperatures.

NMR Spectra

NMR spectra were obtained on bicine solutions that were 0.05–0.2 M in Mg^{2+}, Ca^{2+}, or Zn^{2+}. DSS (2,2-dimethyl-2-silapentane-5-sulfonate, 0.0 ppm to $(CH_3)_4Si$) was employed as an internal standard. Experiments varying the spin rate (rps) were performed to establish which peaks (if any) were sidebands of the HDO resonance (4.8 ppm to DSS).

ESR Spectra

Electron spin resonance spectra on 1:1 and 2:1 bicine to Cu(II) solutions (~10^{-3} M) were recorded on a Bruker ER 200 D SRC spectrometer at various **a** values at ambient temperatures. The relative concentrations of the two Cu(II) complexes were determined *via* computer simulation (ASPECT 2000). Using linewidths and line separations of given spectra as inputs, composite spectra were generated for several different ratios of the two Cu(II) species. The final, relative concentrations were determined *via* a visual comparison of the observed spectra with those simulated. The overall accuracy of the relative concentrations was assessed to be ~5%.

RESULTS

Metal Chelate Binding Constants

Potentiometric formation curves of 2:1, bicine to Mg(II), Ca(II), Mn(II), Zn(II), Co(II), Ni(II), and Cu(II) as well as 1:1, bicine to Cu(II) are shown in Figure 1. Although the actual data yield a more complicated series of graphs, certain lines have been condensed in the interest of clarity. The formation constant values in Table I, however, will allow one to imagine two closely spaced lines, *e.g.*, Mg(II) and Ca(II), where only one line is shown. The ligand curve (L) has a single inflection at **a** = 1.0, corresponding to the removal of the zwitterionic proton of bicine (*1*). It

$$^{-}OOCCH_2NH^{+}(CH_2CH_2OH)_2 \rightleftharpoons {}^{-}OOCCH_2N(CH_2CH_2OH)_2 + H^{+} \qquad (1)$$

was necessary to add 1 equivalent of HCl to a bicine solution in order to determine the protonation constant of the carboxyl group (*2*). The logarithms of the

$$H^{+} + {}^{-}OOCCH_2NH^{+}(CH_2CH_2OH)_2 \rightleftharpoons HOOCCH_2NH^{+}(CH_2CH_2OH)_2 \qquad (2)$$

protonation constant values for the carboxylate and nitrogen were determined to be 1.78 ± .02 and 8.39 ± .01, respectively, which are consistent with literature values.[6]

TABLE I
Metal ion formation constants of bicine.

	Bicine[a]		Glycine[b]	
Metal Ion	log K_1	log K_2	log K_1	log K_2
H^+	8.39 ± .01	1.78 ± .02	9.57	
Mg^{2+}	1.80 ± .03		2.22	
Ca^{2+}	2.66 ± .01		1.39	
Mn^{2+}	3.07 ± .01	2.25 ± .02	2.80	1.92
Co^{2+}	5.30 ± .01	3.38 ± .01	4.64	3.82
Zn^{2+}	5.37 ± .01	3.20 ± .01	4.96	4.23
Ni^{2+}	6.42 ± .01	4.32 ± .01	5.78	4.80
Cu^{2+}	8.07 ± .01[c]	5.40 ± .02[d]	8.15	6.88

[a] 25.0°C and μ = 0.10 M (KNO_3 or $N(CH_3)_4NO_3$). [b] Values from reference[6]. [c] $CuL \rightleftharpoons Cu(H_{-1}L) + H^+$, log $K_{1a} = -7.09 \pm .01$; $Cu(H_{-1}L)(H_2O) \rightleftharpoons Cu(H_{-1}L)OH^- + H^+$, log $K_{OH} = -10.40 \pm .02$. [d] $CuL_2 \rightleftharpoons Cu(H_{-1}L) + L^- + H^+$, log $K_d = -12.36 \pm .02$; $Cu(H_{-1}L)(H_2O) \rightleftharpoons Cu(H_{-1}L)OH^- + H^+$, log $K_{OH} = -10.42 \pm .02$.

Of the 2:1, bicine to M(II), formation curves only Cu(II) shows two distinct low pH buffer zones with inflections at **a** = 0.5 and 1.0, corresponding to the formation of 1:1 (*3*) and 2:1 (*4*) bicine metal complexes.

$$M^{2+} + L^{-} \rightleftharpoons ML^{+} \qquad (3)$$

$$ML^{+} + L^{-} \rightleftharpoons ML_2 \qquad (4)$$

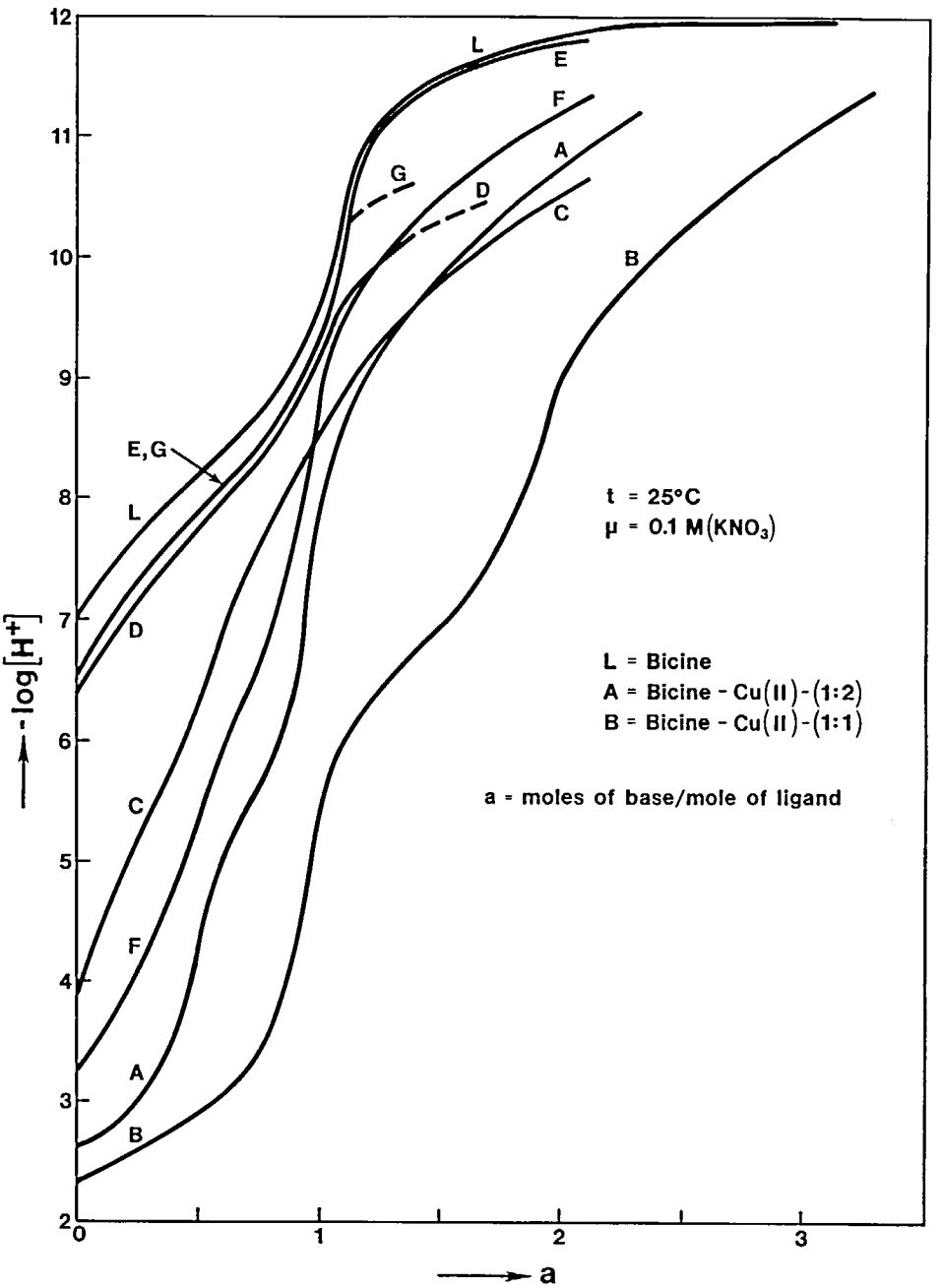


FIGURE 1 Potentiometric formation curves for bicine (L), 1 : 1 bicine to Cu(II) (B) and 2 : 1 bicine to Cu(II) (A), Co(II) (C), Zn(II) C, Mn(II) (D), Ca(II) (E), Ni(II) (F), and Mg(II) (G).

A third high pH buffer zone was shown (a previous communication)[7] to be due to the following dechelation (*5*) and hydroxo complex formation (*6*) reactions.

$$Cu(bicine)_2 \rightleftharpoons Cu(H_{-1}\ bicine)H_2O + bicine^- + H^+ \quad (5)$$

$$Cu(H_{-1}\ bicine)H_2O \rightleftharpoons Cu(H_{-1}\ bicine)OH^- + H^+ \quad (6)$$

The formation curves for 2:1, bicine to Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+} and Ni^{2+} consist of a single low pH buffer zone followed by an inflection at **a** = 1.0. Mn(II), Co(II), Ni(II), and Zn(II) were found to form 1:1 and 2:1 complexes, (*3*) and (*4*), respectively. Mg(II) and Ca(II) were found to form only 1:1complexes (*3*). The constants for all of the above reactions are listed in Table I.

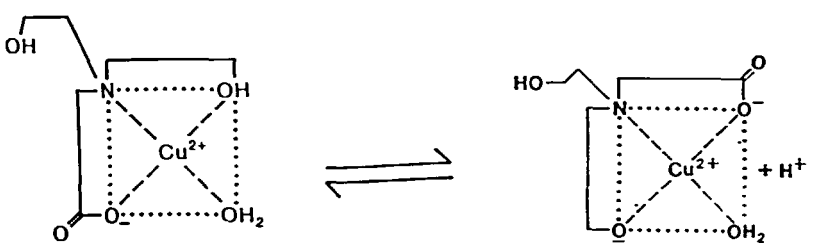
The 1:1 bicine to Mg(II), Ca(II), Mn(II), Co(II), Ni(II), and Zn(II) formation curves are not shown and consist of a single buffer region terminated by an inflection at **a** = 1.0, followed by precipitation of metal hydroxides or oxides with the exception of Ca(II). The 1 : 1 bicine to Cu(II) curve is more interesting, (Figure 1) with inflections at **a** = 1.0 (acidic), 2.0 (neutral), and 3.0 (basic), corresponding to reactions in (*3*), (*7*) and (*6*), respectively, where

$$CuL^+ \rightleftharpoons Cu(H_{-1}L) + H^+ \quad (7)$$

$H_{-1}L$ represents bicine with a coordinated, ionized hydroxyl group.

Visible Spectra

Visible spectral data for the 1:1 bicine to Cu(II) solutions at various **a** values are listed in Table II. From **a** = 0 to 1, there is a small monotonic shift from 807 to 761 nm, corresponding to the formation of CuL^+. From **a** = 1.0 to 2.0 there is a monotonic shift to higher energy (761 to 717 nm) as well as a large increase in ε_{max}, which is attributed to coordination of an ionized hydroxyl group (I), (*8*).



(*8*)

I

Ionized hydroxyl groups are strong σ-donors and cause a large shift to higher energy. From **a** = 2.0 to 3.0, there is little change in λ_{max} (717 nm to 724 nm) or ε_{max} which is typical of hydroxo complex formation (*6*). OH^- is a slightly poorer σ-donor than H_2O.

Visible spectra for 2:1, bicine to Cu(II) solutions, are shown in Figure 2. The λ_{max} values at **a** = 0.5, 1.0, and 2.0 are 753, 611, and 724 nm, respectively. At **a** = 0.5, $CuL^+$is formed, at **a** = 1.0, CuL_2 is formed, and at **a** = 2.0, $[Cu(H_{-1}L)OH^-]$ is present. The λ_{max} and ε_{max} values at **a** = 1.0 are consistant with a wide variety of bisaminoacidate copper(II) complexes,[8] while those at **a** = 2.0 are identical to those

of a solution of 1:1 bicine to Cu(II) at a = 3.0 (see Table II), indicating dechelation of a bicine chelate upon alcohol group ionization and coordination (*5*).

The 1:1 and 2:1 visible spectral data for bicine toNi(II) and Co(II) are listed in Table II. The spectra are unremarkable and show monotonic shifts to higher energy as the 1:1 and 2:1 complexes form. There is no change in stereochemistry as the complexes remain octahedral.

TABLE II
Visible spectra of bicine–metal ion solutions.[a]

	Co(II)		Ni(II)		Cu(II)	
a values	1:1	1:2	1:1	1:2	1:1[b]	1:2
0	509(5)	508(5)	655(2),392(5)	657(2),388(6)	775(21)	767(25)
0.50	506(7)	502(7)	653(3),387(6)	631(3),385(7)	767(24)	753(26)
1.0	506(8)	496(6)	638(3),381(7)	588(4),370(7)	761(27)	611(33)
1.5					716(47)	600(47)
2.0					717(67)	724(64)

[a] = λ_{max} in nm (ε_{max} in $cm^{-1}\ M^{-1}$). [b] a = 3.0, 724(64).

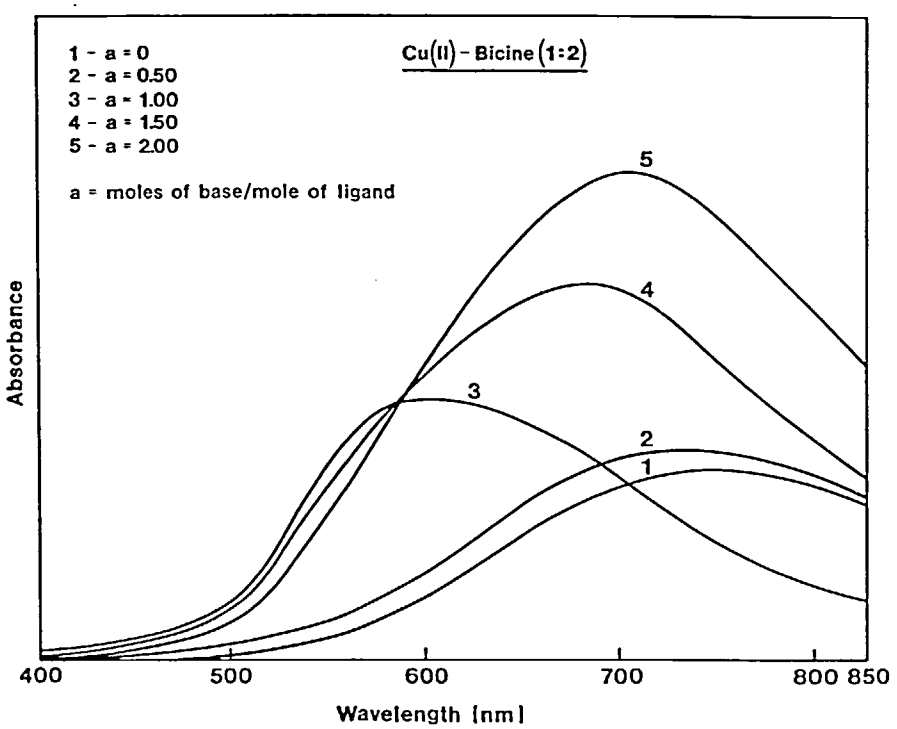
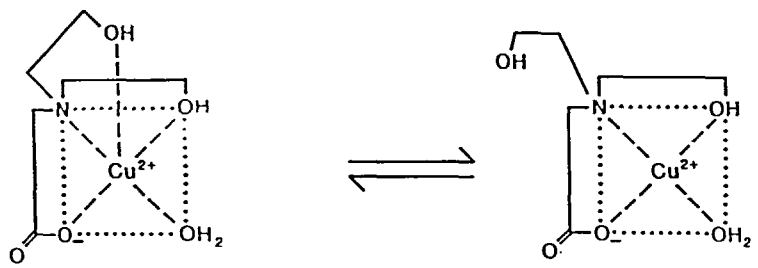


FIGURE 2 Visible spectra of 2:1 bicine to Cu(II) solutions.

ESR Spectra

The ESR spectra have been published previously[7] and were used along with potentiometric and visible spectral data to verify (*5*), dechelation of an aminoacidate ligand upon coordination of an ionized hydroxyl group. The ESR spectra of 1:1 and 2:1 bicine to Cu(II) solutions have identical "g" values at a = 3.0 and 2.0, respectively, indicating that the same Cu(II) complex exists in solution, [Cu-$(H_{-1}L)OH^-$]. The ESR spectra at a = 1.0 for both 1:1 and 2:1 bicine to Cu(II) solutions have very different "g" values indicating the formation of CuL^+ and CuL_2, respectively. The ESR spectra of both 1:1 and 2:1 solutions at a = 1.0 and 0.5, respectively, clearly indicate that two species are present at the inflection point for 1:1 bicine to Cu(II) chelate formation. Both spectra are identical and contain five lines. Computer analysis was consistent with two species having "g" values (percentages) of 2.1667 (85%) and 2.1324 (15%). Since these spectra were taken at inflection points of the formation curves, the equilibrium should be proton independent. The following equation, (*9*), involving coordination of an alcohol group is consistent with potentiometric, visible, and ESR data. Remarkably similar 5 line spectra were observed in the 1:1 *N*-(2-acetamido)imminodiacetic acid (ADA)[10] to Cu(II) and *N,N*-bis(carboxymethyl)glycylglycine (CMGG)[11] to Cu(II) complexes. The latter metal complexes have a nitrogen and two carboxylate donors in equatorial sites of Cu(II), and an equilibrium amount of coordination to an axial site of Cu(II) by an oxygen of the amide group of ADA and of the peptide group of CMGG.



(9)

NMR Spectra

Proton NMR data for bicine and for 1:1 and 2:1 bicine to Mg(II), Ca(II), and Zn(II) solutions at various a values are summarized in Table III. Assignments were based on B being the singlet. The triplet at 3.97 ppm

$$^-OOCCH_2ND^+(CH_2CH_2OH)_2$$

B A C

(a = 0, bicine) was assigned to C due to its small shift to 3.76 ppm upon deprotonation of the nitrogen whereas the CH_2 group (A) adjacent to the nitrogen exhibited a larger shift (3.50 ppm to 2.90 ppm). As is typical in such systems, the removal of the zwitterionic proton of bicine resulted in all three resonances moving upfield (DDS as the standard), due to the loss of the positive charge on N.

The 1:1 complexes of bicine to Mg(II), Ca(II), and Zn(II) all have absorbances at higher ppm values than does the bicinate ion, which is usually attributed to the

TABLE III
NMR spectra data for bicine and bicine–metal ion solutions.

	Bicine(L)			1:1 L to Mg^{2+}			2:1 L to Mg^{2+}			1:1 L to Ca^{2+}			2:1 L to Ca^{2+}		
a value	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
0	3.50	3.92	3.99	3.50	3.90	3.97	3.50	3.89	3.98	3.52	3.90	3.99	3.50	3.90	3.99
0.5	3.15	3.58	3.85	3.20	3.66	3.90	3.15	3.62	3.86	3.28	3.70	3.92	3.35	3.80	3.95
1.0	2.75	3.25	3.70	2.90	3.36	3.76	2.84	3.32	3.72	2.80	3.34	3.82	2.76	3.32	3.78

	1:1 L to Zn^{2+}			2:1 L to Zn^{2+}		
	a	b	c	a	b	c
0	3.50	3.90	3.95	3.50	3.90	3.94
0.5	3.32	3.68	3.93	3.32	3.70	3.92
0.75	3.20	3.59	3.88	3.23	3.65	3.90
1.0	3.10	3.50	3.85	ppt formation		

positive charges of the metal ions. The largest shifts, relative to the bicinate ion, occur for the Zn(II) complex which is probably due to Zn(II) forming a more stable complex than either Mg(II) or Ca(II). It is interesting to note that the relative shift of the α protons (A) is larger for the Mg(II) complex than the Ca(II) chelate, while the relative shift of the protons adjacent to the hydroxyl groups is larger for the Ca(II) complex than the Mg(II) one. The higher charge to size ratio of Mg(II) relative to Ca(II) is probably responsible for the first observation, *i.e.*, assuming both metal ions bind to the nitrogen and carboxylate group. The second observation indicates that one or both hydroxyl groups are coordinated to Ca(II) but not to Mg(II). The small size of Mg(II) relative to Ca(II) usually results in Mg(II) forming more stable complexes with simple amino acids than Ca(II);[6] however, the larger Ca(II) forms more stable complexes with tetradentate chelating agents, *e.g.*, nitrilotriacetic acid, than does Mg(II).[6] Mg(II) is apparently too small to accommodate the "bite" required by the larger chelating agents.

The 2:1 bicine to Mg(II) and Ca(II) solution spectra indicate that both ions form only 1:1 complexes and not 2:1 complexes in agreement with the potentiometric data. All the resonances of the 2:1 ligand to M(II) complexes at $\mathbf{a} = 1.0$ lie about half-way between that of ML^+ and uncoordinated bicinate ion. The rapid ligand exchange rates of Mg(II) and Ca(II), relative to the nmr time scale, result in single sets of absorbances which are fairly broad and situated about half-way between those of the 1:1 ligand to M(II) complex and free ligand. The 2:1 bicine to Zn(II) solution unfortunately yielded a precipitate at $\mathbf{a} = 1.0$.

DISCUSSION

Metal Chelate Formation Constants

The formation constants for 1:1 and 2:1 bicine to M(II) complexes (Table I) are remarkably similar to those reported by Martell and co-workers[9] in 1953 with the exceptions of Ca(II) which was not determined and the hydrolyses and dechelation reactions of the Cu(II) chelates. These latter reactions were unknown at that time. The relative stabilities of the 1:1 and 2:1 metal complexes follow the Irving–Williams series, *i.e.*, Mn(II) < Co(II) ~ Zn(II) < Ni(II) < Cu(II). From a biochemical viewpoint the most important number is the rather large value for the Ca(II) chelate, $10^{2.66}$; this number is large enough that a buffer solution prepared with bicine could extract "labile" Ca(II) from proteins, thereby altering their structures and concomitantly their biological activities.

Martell[9] proposed that one or both of hydroxyl groups of the ligand were bound to the metal ions as well as the nitrogen and oxygen of the amino acid moiety. This premise[9] was based on the fact that the corresponding formation constants of 1:1 M(II) to glycine complexes were all smaller (except Cu(II), the same, and Mg(II), larger), than those of bicine. Since the nitrogen of bicine is a good deal less basic (pK_a, 8.39) than that of glycine (pK_a, 9.57), bicine should form less stable chelates than those of glycine if the alcohol groups were noncoordinating. Since the formation constants were larger or the same (Cu(II)), bicine relative to glycine, alcohol group coordination was indicated. However, no spectroscopic data were proffered; at that time NMR and ESR instruments were unavailable, and few spectrophotometric data existed.

An examination of the stability constant data presented here for the 1:1 bicine to

Mg(II) and Ca(II) chelates supports Martell's premise.[9] Mg(II) forms a more stable chelate with glycine ($10^{2.22}$)[6] than does Ca(II) ($10^{1.39}$); however, bicine forms a more stable 1 : 1 chelate with Ca(II) ($10^{2.66}$) than with Mg(II) ($10^{1.80}$). The low formation constant for [Mg(bicinate)$^+$] is expected if little or no alcohol group coordination occurs. On the other hand, the enhanced stability of [Ca(bicinate)$^+$] relative to [Ca(glycinate)]$^+$ can only be attributed to alcohol group coordination. The above argument is supported by the nmr data as discussed above.

An often useful rule of thumb[12] for octahedral metal complexes is that if the difference (Δ) between log K_1 and log K_2 formation constant values is $\sim$0.8 log units, the chelating agent is probably acting as a bidentate amino acid (glycine, methionine, ethionine) while a Δ of $\sim$1.8 log units indicates a terdentate chelate (iminodiacetic (IMDA) and iminodipropionic acids). The Δ values for the bicine chelates of Co(II), Ni(II), Zn(II), and Mn(II) are 1.9, 2.1, 2.1, and 0.8, respectively, indicating that alcohol group coordination occurs in the Co(II), Ni(II), and Zn(II) complexes but perhaps not in those of Mn(II). The Δ values for Cu-glycine, and Cu-bicine complexes are 1.3 and 2.9, respectively, which indicates that alcohol group coordination does occur with Cu(II). This latter conclusion is in agreement with the esr data presented earlier.

Visible Spectra

The visible spectra of the 1 : 1 and 2 : 1 bicine to Co(II) and Ni(II) complexes are similar to the corresponding metal chelates of glycine; *e.g.*, [Ni(glycine)$^+$] (λ_{max} = 650 nm)[13], [Ni(alanine)$^+$] (λ_{max} = 640 nm)[13] are similar to [Ni(bicine)$^+$] (λ_{max} = 638 nm)]. This is expected in that a coordinated water molecule will have a similar donor strength to a coordinated alcohol group. However, [Cu(bicinate)$^+$] (λ_{max} = 761 nm) absorbs at considerably lower energy than does [Cu(glycinate)$^+$] (λ_{max} = 725 nm).[13] This is in agreement with the ESR data which indicated axial coordination of an alcohol group to Cu(II) (*8*). Coordination of a chelate arm to a Cu(II) axial site usually results in a red shift; this is the well-known pentammine effect. If no axial coordination occurred, one would expect [Cu(bicinate)$^+$] to absorb at $\sim$725 nm, *c.f.*, [Cu(glycinate)$^+$].[13] The effects of axial coordination to Cu(II) on hydroxo complex formation, catalytic activity, and λ_{max} values have been discussed elsewhere.[14]

In summary, the stability constant values obtained here are very similar to those reported by Martell and co-workers[9] and not to other more recent literature values.[6] Martell's premise[9] of alcohol group coordination in bicine–metal complexes is supported by ESR (Cu(II)), NMR (Ca(II), Zn(II)), and visible data (Cu(II)). There appears to be little if any alcohol group coordination to Mg(II) (NMR). Although visible spectra were not, as expected, helpful in discerning whether or not alcohol group coordination to Ni(II) and Co(II) occurs, the stability constant data indicate strongly that it does occur. The data for alcohol group coordination to Mn(II) are conflicting; [Mn(bicinate)$^+$] has a higher log K_1 value than [Mn(glycinate)$^+$], but the Δ values (log K_2 − log K_1) for glycine and bicine are similar.

From a biomedical viewpoint, bicine is *not* a buffer of choice for *in vitro* studies. Not only does bicine form rather stable 1 : 1 complexes with Cu(II) ($10^{8.15}$), Ni(II) ($10^{6.4}$), and Ca^{2+} ($10^{2.66}$), but Cu(II) undergoes further reactions, (*5*) and (*7*), which buffer not only H^+ but also Cu(II). Since the initial report in *Science*,[2] we have received communications concerning the alteration of the chemistry of perfused rat livers (Fe(III)/Fe(II) interferences)[15] and of the failure of sea algae to grow in the

presence of bicine buffer solutions.[16] In the latter case there was an apparent inhibition of urease, a Ni(II) enzyme, as evidenced by the failure of the algae to generate NH_3. The system was well behaved in glycylglycine buffer solutions. The most curious result from an inorganic viewpoint was that after a certain length of time, in the presence of bicine buffer solutions, ammonia production by the algae did resume. Did the algae release or bring to their cell surfaces an enzyme which destroyed [Ni(bicine)$^+$]? Or perhaps did the algae bring to their cell surfaces a specially designed chelating moiety for Ni(II) binding and extract Ni(II) from [Ni(bicine)$^+$] or [Ni(bicinate)$_2$]? In any case the organism overcame the high stability of the Ni-bicine metal chelates.

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