and 0.2 g of **5**. From the mother liquors small amounts of an additional complex, mp 175–180°, were isolated (complex **6**).

Anal. Calcd for $C_{19}H_{16}FeO_3$: C, 65.54; H, 4.63; O, 13.79; Fe, 16.04; mol wt, 348.2. Found: C, 65.30; H, 4.82; O, 13.41; Fe, 17.05; mol wt, 330 (osmometry in $CHCl_3$).

Oxidative Degradation with $FeCl_3$. To a solution of 25 g of anhydrous $FeCl_3$ in 100 ml of acetonitrile, a concentrated acetonitrile solution of 5 g of complex **1** was added over a period of 30 min at room temperature. The CO evolved during this time amounted to 41.7 mmoles (97% of the theoretical amount). The acetonitrile solution was evaporated to dryness at 20° and reduced pressure. The residue was extracted with 150 ml of cold methylene chloride. Vacuum evaporation of the methylene chloride solution afforded 0.32 g (25%) of colorless crystals which were sublimed and identified as naphthalene by comparison of the infrared spectrum and the determination of the mixture melting point with an authentic sample. Similar yields of naphthalene were obtained from the degradation of complex **2**. Naphthalene was also isolated from complexes **3**, **4**, and **5**, albeit in smaller yields.

Characteristic infrared bands of complexes **1–5**, **8**, and **10** (KBr, in cm^{-1}; CO = metal carbonyl) follow.

1. 3040, 2940, 2918, 2045 (CO), 1976 (CO), 1960 (CO), 1930 (sh), 1649, 1450, 1399, 1376, 1349, 1252, 1238, 1227, 1180,...

2. 3130, 2951, 2045 (CO), 1977 (CO), 1960 (CO), 1630, 1450, 1346, 1300, 1250, 1166, 1130,...

3. 2941, 2067 (CO), 2044 (CO), 2025 (CO), 1989 (CO), 1720, 1637, 1450, 1404, 1349, 1312, 1296, 1269, 1230,...

4. 2950, 2045 (CO), 1980 (CO), 1980 (CO), 1955 (CO), 1465, 1450, 1401, 1370, 1339,....

5. 2941, 2057 (CO), 2041 (CO), 1984 (CO), 1960, 1950, 1450,....

8 (TCNE adduct of **1**). 3020, 2940, 2370, 2265, 2181, 2125 (CO), 2046 (CO), 1972 (CO), 1459, 1399, 1379, 1350, 1330, 1288, 1258, 1191,....

10. 3030, 2919, 2084 (CO), 2044 (CO), 1993 (CO), 1648, 1460, 1406, 1359, 1317, 1310,....

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The Reaction of L-Carnosine with Cobalt(II)[1]

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Abstract: The temperature-jump method has been used to determine the complexation rate constants for the reaction of L-carnosine with cobalt(II) at 25° and ionic strength 0.1 *M*. The association rate constant is consistent with a mechanism in which release of a water molecule from the metal ion's inner coordination sphere is rate determining. The rate constants for the reaction, $Co^{2+} + HL \rightleftharpoons CoHL^{2+}$ (where HL represents the neutral form of the ligand), are $k_1 = 4.2 \times 10^5$ (±20%) M^{-1} sec^{-1} and $k_{-1} = 87$ (±20%) sec^{-1}. Since the cobalt complex is a seven-membered ring system, the results are taken as evidence that the ligand entropy contribution to steric effects in fast metal-complex substitution reactions is considerably less significant than the ring-strain contribution.

Many transition metal complexation reactions have been studied by fast-reaction techniques. The conclusion, which has been reached on the basis of the experimental data, is that these reactions require at least two steps.[3] First, there is a diffusion-limited ion-pair formation between the aquated metal ion and the reactant ligand—a process which may, itself, involve more than one discrete step. Second, and rate determining, is the substitution of the reactant ligand into the inner coordination shell. For most systems, the slowest or rate-determining part of this second step is the release of an inner-coordination-sphere water molecule from the fully aquated metal ion. This reaction is characteristic of the metal and is not affected by the ligand.

Recent studies of the reactions of cobalt(II) with β-alanine and β-aminobutyric acid have shown that, for these systems, the association rate constants are ligand dependent.[4,5] These observations were accounted for by demonstrating that the rate of complex formation becomes a characteristic of the ligand when control of the rate passes from the release of a water molecule coordinated in the metal ion's inner hydration sphere to a sterically hindered formation of a metal chelate ring system. The bidentate ligands β-alanine and β-aminobutyric acid form six-membered rings with metal ions rather than the five-membered rings formed by most other chelating ligands. The anomalously slow complexation rates were postulated to be the result of a ring-strain effect and an entropy-loss effect when forming a six-membered chelate ring as compared to a five-membered ring. A thermodynamic study of metal binding with amino acids and diamine bases has demonstrated that, when chelate ring size increases beyond five members, the stability of the complex formed decreases.[6] Although part of this effect could be accounted for on the basis of a chelate entropy effect,[7] the magnitude of the destabilization

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[3] M. Eigen and R. G. Wilkins in "Mechanisms of Inorganic Reactions," Advances in Chemistry Series, No. 49, American Chemical Society Washington, D. C., 1965.

[4] K. Kustin, R. F. Pasternack, and E. M. Weinstock, *J. Am. Chem. Soc.*, **88**, 4610 (1966).

[5] A. Kowalak, K. Kustin, R. F. Pasternack, and S. Petrucci, *ibid.*, **89**, 3126 (1967).

[6] H. Irving, R. J. P. Williams, D. J. Ferrett, and A. E. Williams, *J. Chem. Soc.*, 3494 (1954).

[7] G. Schwarzenbach, *Helv. Chim. Acta*, **35**, 2344 (1952).

is considerably larger than the value calculated from entropy considerations alone. An analogous argument has been developed for the kinetics of these reactions.[4]

In view of the fact that the kinetic behavior of cobalt(II) with regard to five- and six-membered rings has already been studied, we are here reporting on the behavior of this ion with regard to the formation of a seven-membered ring as in the complex with L-carnosine. The results of these kinetic studies on the seven-membered cobalt–carnosine complex shed further light on the relative importance of the ring-strain contribution and the entropy contribution to the over-all steric effect.

L-Carnosine (β-alanyl-L-histidine) is a dipeptide found in muscle and organs such as the kidney and pancreas of many vertebrates.[8] Knowledge of its occurrence has stimulated much research into the elucidation of its metabolic role. For example, it had early been reported that carnosine might be effective in stimulating muscle contraction.[9] Inquiries into the nature of the carnosine function along these lines took on a different direction when Jencks and Hyatt[10] demonstrated that any effect of carnosine on glycolytic, oxidative, or other processes was abolished when carnosine was incubated with EDTA. This finding, as well as the discovery of a metal-stabilized enzyme which attacks the peptide bond in carnosine,[11] disclosed the close association between metal ions and carnosine. The important thermodynamic properties of aqueous solutions of carnosine and carnosine complexes have been determined by Lenz and Martell.[12a] Those of their findings relevant to this study, as well as some other pertinent equilibrium constants, are listed in Table I.

Table I. Stoichiometric Equilibrium Constants[a] at Ionic Strength 0.1 *M* and 25°

$K^a{}_1$	$= [H^+][HL]/[H_2L^+] = 1.74 \times 10^{-7}$
$K^a{}_2$	$= [H^+][L^-]/[HL] = 4.37 \times 10^{-10}$
$K^{Co}{}_{CoHL}$	$= [CoHL^{2+}]/[Co^{2+}][HL] = 4.90 \times 10^3$
K_{HIn}	$= [H^+][In^-]/[HIn] = 1.26 \times 10^{-8}$ [b]

[a] All carnosine and carnosine–metal constants are from ref 12a. [b] I. M. Kolthoff, *J. Phys. Chem.*, **34**, 1466 (1930); HIn = phenol red.

Experimental Section

Fisher reagent grade nitrate salts of potassium(I) and cobalt(II) were used. Nutritional Biochemicals Corp. L-carnosine was likewise used without further purification. The indicator introduced to follow the course of the reaction was Aldrich reagent grade phenol red.

The temperature-jump apparatus has been described elsewhere.[13] Solutions were made up with requisite amounts of metal ion added to dissolved ligand and indicator. The ionic strength was brought to 0.1 *M* with KNO_3 and the pH was adjusted by the dropwise addition of dilute NaOH and/or HNO_3. The temperature was 25 ± 1°.

Test solutions of either metal ion or ligand in the absence of the other showed no discernible relaxation effects. Each relaxation time represents an average of at least three photographic determinations. The relative error for these determinations is ±10%.

Results and Treatment of Data

The reaction under study is an example of a metal–ligand complexation. The ligand is dibasic, however, and exists in solution in at least three forms differing in their degree of protonation. Moreover, there are also other species present. For example, the extent of the reaction is followed by a pH indicator, HIn, coupled to the ligand. Hence, the following protolytic equilibria

$$H_2L^+ \rightleftharpoons H^+ + HL$$

$$HL \rightleftharpoons H^+ + L^-$$

$$HIn \rightleftharpoons H^+ + In^-$$

(where the symbol HL refers to the neutral, or zwitterion form of carnosine) must always be considered in deriving the relaxation expressions for the metal ion. However, these protolytic reactions can be assumed to reach equilibrium far more rapidly than reactions involving metal or metal complex. All the pertinent equilibrium constants used in this study are given in Table I. Charges are indicated in this table, but they will be omitted in the remainder of the article, unless otherwise specified.

The reaction of interest is

$$\mathrm{Co} + \mathrm{HL} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{CoHL} \qquad (1)$$

The equilibrium quotient

$$K^{Co}{}_{CoHL} = k_1/k_{-1} \qquad (2)$$

has been previously determined for this system (*cf.* Table I). Use of the mass action expression and the conservation equations (3) (in which δ designates the

$$\begin{aligned} \delta[Co] &= \delta[H_2L] + \delta[HL] + \delta[L] = -\delta[CoHL] \\ \delta[HIn] &= \delta[L] - \delta[H_2L] - \delta[H] = -\delta[In] \end{aligned} \qquad (3)$$

deviation from equilibrium of the variable under consideration) in the linearized rate equation for (1) leads to the complete reciprocal relaxation time expression

$$\frac{1}{\tau} = k_1\left(\frac{[Co]}{1 + \alpha + \alpha'} + [HL]\right) + k_{-1} \qquad (4)$$

in which

$$\alpha = \frac{\delta[H_2L]}{\delta[HL]} = \frac{[H]^2 + \beta[H][L] + \beta K^a{}_2[HL]}{K^a{}_1[H] + K^a{}_1\beta[L] + \beta[H][HL]} \qquad (5)$$

$$\alpha' = \frac{\delta[L]}{\delta[HL]} = \frac{K^a{}_1K^a{}_2 + \beta[H][L] + \beta K^a{}_2[HL]}{K^a{}_1[H] + K^a{}_1\beta[L] + \beta[H][HL]} \qquad (6)$$

where

$$\beta = \frac{K_{HIn} + [H]}{K_{HIn} + [In] + [H]} \qquad (7)$$

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(8) L. Prahl, *Ernaehrungsforschung*, **9**, 60 (1964).

(9) M. C. Goodall, *Nature*, **178**, 539 (1956), and references therein.

(10) W. P. Jencks and M. Hyatt, *Biochim. Biophys. Acta*, **31**, 262 (1959).

(11) H. T. Hanson and E. C. Smith, *J. Biol. Chem.*, **179**, 789 (1949).

(12) (a) G. R. Lenz and A. E. Martell, *Biochemistry*, **3**, 750 (1964). Earlier work on this system was reported by R. B. Martin and J. T. Edsall, *J. Am. Chem. Soc.*, **82**, 1107 (1960), who interpreted their potentiometric data somewhat differently than have Lenz and Martell. However, the more recent work makes a strong argument for the formation of a seven-membered ring species, and the kinetic data for the copper–carnosine system (*cf.* ref 12b) are consistent with this suggestion. (b) R. F. Pasternack and K. Kustin, *ibid.*, **90**, 2295 (1968).

(13) P. Hurwitz and K. Kustin, *Inorg. Chem.*, **3**, 823 (1964).

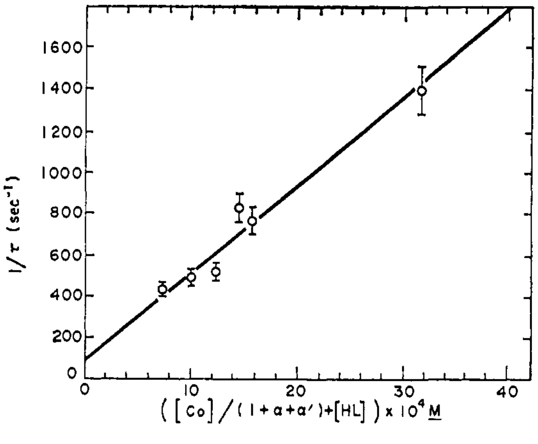

Figure 1. A plot of $1/\tau$ *vs.* ([Co]/(1 + α + α') + [HL]).

If only one protolytic dissociation need be considered, then expressions 5 and 6 may be simplified by setting $K^a{}_2 = 0$ and $[L] = 0$. Thus

$$\lim_{K^a{}_2[L]\to 0}\left(\frac{\delta[H_2L]}{\delta[HL]}\right) = \frac{[H]}{K^a{}_1 + \beta[HL]}$$

and

$$\lim_{K^a{}_2[L]\to 0}\left(\frac{\delta[L]}{\delta[HL]}\right) = 0$$

in exact agreement with the equations previously derived by Hammes and Steinfeld.[14] Explicit calculations of the quantities α and α' led to $0 \leq (\alpha + \alpha') \leq 0.03$.

In solutions containing polyprotic chelating agents, it is always possible to form complexes by reactions such as the following (including charges for the sake of clarity).

$$Co^{2+} + H_2L^+ \rightleftharpoons CoHL^{2+} + H^+ \qquad (8)$$

This step is kinetically distinguishable from reaction 1. Its inclusion in the mechanism results in additive hydrogen-ion-dependent terms in the right-hand side of (4). Observation of this type of dependence has been previously noted.[15] That no such dependence is exhibited by this system indicates that, within experimental error, complexation *via* reaction with H_2L^+ is kinetically insignificant in the pH range studied.

It is to be expected from eq 4 that a plot of $1/\tau$ *vs.* ([Co]/(1 + α + α') + [HL]) should result in a straight line. Such a plot is shown in Figure 1; the slope of the line yields $k_1 = 4.2 \times 10^5$ (±20%) M^{-1} sec^{-1}, whereas the intercept results in $k_{-1} = 87$ (±20%) sec^{-1}. The ratio of these numbers is

$$K = \frac{k_1}{k_{-1}} = \frac{4.2 \times 10^5\ M^{-1}\ \text{sec}^{-1}}{87\ \text{sec}^{-1}} = 4.8 \times 10^3\ M^{-1}$$

in excellent agreement with the value of 4.9×10^3 M^{-1} obtained by Lenz and Martell. The experimental conditions and a summary of the observed relaxation times are shown in Table II. The calculated relaxation times are shown for comparison.

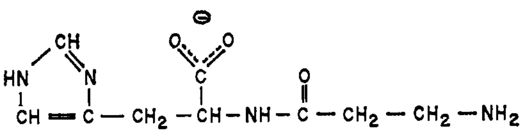

Figure 2. The anionic form of L-carnosine.

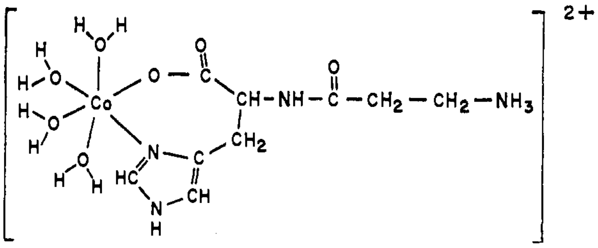

Figure 3. The cobalt(II)–carnosine complex.

Table II. Relaxation Spectra of Cobalt(II)–L-Carnosine Solutions[a]

[L-Carnosine]$_0$ × 10^3	[Co^{2+}]$_0$ × 10^3	[H$^+$]	τ_{exptl} × 10^3, sec	τ_{calcd} × 10^3, sec
0.999	1.04	7.24×10^{-8}	2.3	2.5
0.787	1.67	3.17×10^{-7}	1.9	1.7
1.41	1.70	1.12×10^{-7}	2.0	2.0
2.77	3.13	8.13×10^{-8}	1.3	1.5
4.19	2.94	9.33×10^{-8}	1.2	1.3
8.15	3.31	1.23×10^{-7}	0.71	0.71

[a] All concentrations are molar. The subscript zero refers to the total stoichiometric concentration. The total concentration of phenol red is 2.0×10^{-5} M in each experiment.

Discussion

The chelating agent L-carnosine is a dipeptide consisting of β-alanine and L-histidine moieties. The molecule (in the negatively charged form) has the structural formula in Figure 2. The formation of a complex between the neutral molecule (in the zwitterion form) and cobalt(II) has been postulated to involve chelation through the imidazole ring of histidine and the carboxyl group.[12a] This chelate complex containing a seven-membered ring is shown in Figure 3.

As outlined in the introductory section, for most complex formation reactions the release of a water molecule from the fully aquated metal ion's inner coordination sphere constitutes the rate-determining step in the over-all process. If, however, the formation of a chelate ring is sufficiently unfavorable sterically, ring closure can become rate determining.[4,5] Since the energies involved are not large, the effect is most important for very labile ions such as cobalt(II). Two terms tend to make the formation of certain six-membered chelate rings slower than the formation of a five-membered chelate ring. One is the additional ring strain in forming a six-membered ring, and the other is the additional entropy loss arising from restricting the motions of four atoms instead of three, which we

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[14] G. G. Hammes and J. I. Steinfeld, *J. Am. Chem. Soc.*, **84**, 4639 (1962).

[15] F. P. Cavasino, *J. Phys. Chem.*, **69**, 4380 (1965).

have termed "ligand entropy loss." The results reported on here may be used to assess the relative importance of these two effects.

Second-order substitution rate constants for cobalt(II) with several ligands are shown in Table III. Comparison with the value of $4.2 \times 10^5\ M^{-1}\ \text{sec}^{-1}$ indicates that, within experimental error, the first association rate constant reported on in this paper has the normal value. The observed second-order rate constant, k_1, for normal substitution is $k_1 = k_0K_a$ where k_0 is the first-order rate constant for release of a water molecule from the inner coordination sphere of the fully aquated metal ion. The factor K_a in the above expression is an equilibrium quotient defined as

Table III. Second-Order Substitution Rate Constants for Cobalt(II) with Several Ligands

$$Co^{2+} + L^{q-} \xrightarrow{k_f} CoL^{(2-q)+}$$

L^{q-}	k_f, M^{-1} sec^{-1}	Ref
Sulfate^{2-}	3×10^6	*a*
Malonate^{2-}	9×10^6	*b*
α-Alanate$^-$	6×10^5	*c*
Glycinate$^-$	5×10^5	*d*
Glycylglycinate$^-$	5×10^5	*d*
Imidazole	1×10^5	*d*
Sterically Controlled Substitution		
β-Alanate$^-$	7×10^4	*c*
β-Aminobutyrate$^-$	2×10^4	*e*

[a] M. Eigen and K. Tamm, *Z. Elektrochem.*, **66**, 107 (1962). (A direct measurement of the first-order rate constant was obtained in this paper using the sound absorption technique. A value of 15 was used for K_a to obtain the second-order rate constant; *cf.* Cavasino, footnote *b*). [b] F. P. Cavasino, *Ric. Sci.*, **35** (II-A), 1120 (1965). [c] K. Kustin, R. F. Pasternack, and E. M. Weinstock, *J. Am. Chem. Soc.*, **88**, 4610 (1966). [d] G. G. Hammes and J. I. Steinfeld, *ibid.*, **84**, 4639 (1962). [e] A. Kowalak, K. Kustin, R. F. Pasternack, and S. Petrucci, *ibid.*, **89**, 3126 (1967).

$$K_a = [M(aq),L]/[M(aq)][L]$$

where the species [M(aq),L] is an outer-sphere ion pair.

By using a suitably calculated value of K_a,[3] the rate constants quoted in Table III have been shown to be consistent with the value of k_0 determined by an nmr technique.[16] A further comparison with the normal values given in Table III indicates that the association between cobalt(II) and neutral L-carnosine involves an effective unit negative charge on the ligand. This conclusion seems reasonable considering the relatively large size and special structure of L-carnosine, in which the positive charge may reside on a site remote from the negatively charged carboxyl group.

Hence, it may be concluded that release of coordinated water is rate determining for L-carnosine with cobalt(II) and that the free energy of activation for this step exceeds that of ring closure. Therefore, the steric effect for L-carnosine is so small that it does not manifest itself for cobalt(II). However, the ligand entropy term proposed by Schwarzenbach[7] increases as ring size increases and, therefore, this entropy term cannot be the dominant contribution to the steric effect. As the chelate ring size increases from six members to seven members, the ring-strain effect would tend to decrease because of greater possibilities for puckering of the ring. To observe a steric effect with a seven-membered ring one must make recourse to a more labile metal ion system such as aqueous copper(II).[12b]

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(16) T. J. Swift and R. E Connick, *J. Chem Phys.*, **41**, 2553 (1964).