Structures of the Copper-Carnosine and Copper-Anserine Chelates

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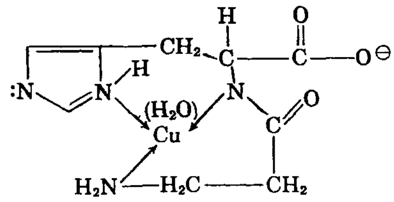
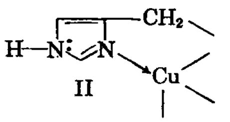
The importance of metal ions in many biochemical reactions is well known. Specifically, the dipeptidases, including carnosinase, require divalent metal ions for their activity. This is due, in part, to the ability of the metal ion to simultaneously coordinate with the substrate and the enzyme. Thus the knowledge of metal dipeptide chelate structures is basic to the elucidation of this phenomenon.

Dobbie and Kermach[1] reported that the most probable structure for the copper chelate of carnosine was I. Recently Martin and Edsall[2] concluded on the basis of potentiometric studies that the structure proposed, I, was essentially correct. However, they postulated that the pyridine nitrogen rather than the pyrrole nitrogen of the imidozole ring is coordinated with the copper, II. Structure II is preferred because the aromatic character of the imidazole ring is retained. Both authors present convincing evidence for the ionization of the peptide hydrogen on formation of the chelate. However, there existed some doubt as to whether the copper was coordinated with the nitrogen or the oxygen of the peptide linkage. The sole basis for the choice was the preferred structure of a six-membered ring produced by the coordination of the amide nitrogen with copper. On the other hand, Tomita,[3] *et al.* reported that in several dipeptide chelates, including copper-carnosine and copper-anserine, copper was coordinated to the carboxylate anion and the carbonyl oxygen of the amide.



I



II

The present investigation was undertaken to establish correct structures for copper-carnosine and copper-anserine from a study of their infrared absorption spectra. Specifically, infrared studies were undertaken to determine whether the carboxylate anion is coordinated to copper in the chelate; and whether chelation to copper involves the nitrogen or the oxygen of the amide linkage. Recently, Rosenberg[4] has elucidated the structures of many amide-metal and dipeptide-metal chelates from a careful examination of their infrared spectra.

The major infrared absorption bands of carnosine, *N*-acetylcarnosine, anserine, copper-anserine, and copper-carnosine are given in Table I.

TABLE I

Compound	$-NH_3^+$	$-\overset{O}{\overset{\|}{C}}-$ (amide)	$-\overset{O}{\overset{\|}{C}}-O^-$
Anserine	1590 cm.$^{-1}$	1657 cm.$^{-1}$	1412; 1570 cm.$^{-1}$
Carnosine	1587 cm.$^{-1}$	1656 cm.$^{-1}$	1411; 1560 cm.$^{-1}$
N-Acetylcarnosine	Absent	1658; 1656 cm.$^{-1}$	1411; 1560 cm.$^{-1}$
Copper-carnosine	Absent	1618 cm.$^{-1}$	1407; 1562 cm.$^{-1}$
Copper-anserine	Absent	1603 cm.$^{-1}$	1415; 1563 cm.$^{-1}$

The absorption bands were assigned on the basis of comparison with reference compounds—*i.e.*, imidazole, histamine-dihydrochloride, histidine, and *N*-acetylhistidine—and previously recorded values[5] for similar compounds. It has been well established[5] that ionized carboxyl has two absorption maxima, 1550 cm.$^{-1}$ and 1410 cm.$^{-1}$ (due to the symmetrical and asymmetrical modes of vibration), and that unionized carboxyl has only one major absorption in the 1700–1750 cm.$^{-1}$ region. Rosenberg[6] has clearly shown that a copper to carboxylate anion coordination results in a shift of the 1550 cm.$^{-1}$ absorption maxima to higher frequencies as a result of the covalent character of the copper to oxygen bond, specifically, sodium glycinate, 1590 cm.$^{-1}$, copper glycinate,1620 cm.$^{-1}$. The presence of unchanged carboxylate absorption maxima in the copper chelates and in anserine and carnosine (Table I) strongly indicates that the carboxylate anion is not involved in the chelate. The absorption due to $-NH_3^+$ in anserine and carnosine is consistent with the fact that all amino acids capable of possessing the $-NH_3^+$ structure and their hydrochlorides show absorptions in the 1500–1600 cm.$^{-1}$ region.[5] The absence of the $-NH_3^+$ absorption in *N*-acetylcarnosine and in the two copper chelates strongly indicates that copper chelates to the primary amino group of anserine and carnosine. The amide carbonyl

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(1) H. Dobbie and O. Kermach, *Biochem. J.*, **59**, 254 (1955).

(2) R. B. Martin and J. T. Edsall, *J. Am. Chem. Soc.*, **82**, 1107 (1960).

(3) M. Tomita, N. Hamamura, H. Tamiya, M. Takehara, and K. Tomita, *Z. physiol. Chemie*, **295**, 128 (1953).

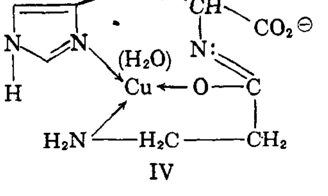
(4) A. Rosenberg, *Acta Chem. Scand.*, **11**, 1390 (1957).

(5) L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, John Wiley and Sons, Inc., New York, N. Y., 1954, p. 203 ff.

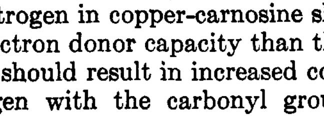
(6) A. Rosenberg, *Acta Chem. Scand.*, **10**, 840 (1956).

Fig. 1. The infrared absorption spectra of: ——— copper-carnosine: ——— carnosine

absorption of carnosine and anserine, which occurs at 1656 and 1657 cm.$^{-1}$, respectively, was no longer present in copper-carnosine and copper-anserine. However, in the copper chelates, absorption maxima were observed at 1618 and 1603 cm.$^{-1}$, respectively (see Fig. 1). Two structures, III and IV, may be postulated from two interpretations consistent with previous work[1,2] and with the infrared data. One is based upon the fact that copper in all of its oxidation states is less electronegative than hydrogen[7]; therefore, the peptide nitrogen in copper-carnosine should have a greater electron donor capacity than that in carnosine. This should result in increased conjugation of the nitrogen with the carbonyl group and consequently should produce decreased C=O double bond character. Thus the absorption maximum at 1603 cm.$^{-1}$ in copper-carnosine (Table I) may be attributed to a shift, to a lower frequency, in the peptide carbonyl absorption. This interpretation favors structure III. The alternate interpretation favors structure IV and is based upon the fact that the conjugated imino group (imino ether type) absorbs in the range 1480–1660 cm.$^{-1}$.[8] Thus the absorption maxima at 1603 cm.$^{-1}$ may be due to the presence of the —C=N— in copper-carnosine.



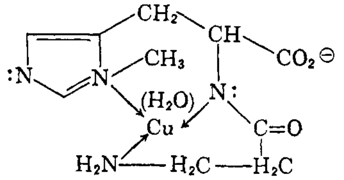
III



IV

On the basis of relative stabilities the two six-membered ring structure of III is preferred to the mixed eight, six-membered structure of IV. However, such mixed ring systems are known, as in IV, in which the six-membered ring lends stability to the chelate.[9] Examination of molecular models indicated that the formation of an eight-six ring fusion, IV, is unlikely if quadricovalent copper is to have a planar configuration.[10] In this respect III is highly preferred.

Similar interpretations can be applied to the anserine and copper-anserine spectra, in which there is a similar absorption maximum shift (Table I). Molecular models revealed that copper in copper-anserine, in contrast to copper-carnosine, must coordinate with the pyrrole nitrogen in order to maintain its planar configuration, resulting in the loss of ring aromaticity. Thus, V, is suggested for the structure of copper-anserine.



V

EXPERIMENTAL

The infrared spectra of the following compounds were determined on a Beckman IR-7 spectrophotometer as solids in potassium bromide pellets.

L-Carnosine and L-histidine were obtained from Mann Research Laboratories.

Imidazole and histamine·dihydrochloride were kindly supplied by Dr. S. Vratsanos of Fordham University.

N-Acetylhistidine was obtained from Nutritional Biochemicals Corporation.

L-Anserine was prepared from tuna muscle by a modified procedure[11] of Jones.[12]

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(7) L. Pauling, *The Nature of the Chemical Bond*, Cornell University Press, Ithaca, N.Y., Third Edition, 1960, p. 93.

(8) L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, John Wiley and Sons, Inc., New York, N. Y., 1954, p. 227 ff.

(9) A. E. Martell and M. Calvin, *Chemistry of the Metal Chelate Compounds*, Prentice-Hall, Inc., N. J., 1952, p. 134 ff.

(10) L. Pauling, *The Nature of the Chemical Bond*, Cornell University Press, Ithaca, N. Y., Third Edition, 1960, p. 158.

(11) A. Lukton and H. S. Olcott, *Food Research*, **23**, 611 (1958).

(12) N. R. Jones, *Biochem. J.*, **60**, 81 (1955).

Copper-carnosine and copper-anserine were prepared by the procedure of Deutsch, *et al.*[13]

N-acetylcarnosine was prepared by the previously described procedure for the preparation of *N*-acetylamino acids.[14] L-Carnosine was refluxed at 100° for 2 hr. with 2 equivalents of acetic anhydride and 10 equivalents of glacial acetic acid. After repeatedly adding water and evaporating to dryness *in vacuo*, the product was crystallized from absolute methanol. It was recrystallized to a constant melting point of 209–210° (uncor.) and gave a negative ninhydrin reaction.

Anal. Calcd. for $C_{11}H_{15}N_4O_4$: C, 49.20; H, 5.97. Found: C, 48.70; H, 5.86.

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[13] A. Deutsch, M. G. Eggleton, and P. Eggleton, *Biochem. J.*, **32**, 203 (1938).

[14] P. J. Fodor, V. E. Price, and J. P. Greenstein, *J. Biol. Chem.*, **178**, 503 (1949).

Thermal Properties of a Series of Copper Bis(β-diketone) Polymers as a Function of the Number of Connecting Methylene Groups

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Preparation of coordination polymers of bis-(β-diketones) have been reported by several investigators.[1,2] In all cases the copper chelate is obtained as a powder. By increasing the length of the methylene chain joining coordination sites, the properties of the polymers might be expected to approach the properties of long chain hydrocarbons. Such changes in properties would result from a decrease in the average bulk of the polymer per unit length and a decrease in the interaction between coordination sites per unit length of polymer. The effect on the thermal properties of this increase in the number of methylene groups in the bridge between complex sites is reported here. The possibility of obtaining a melt before decomposition occurs was investigated by differential thermal analysis, and visual observation of the copper(II) chelates of bis(β-diketones) of the type $C_6H_5COCH_2CO(CH_2)_nCOCH_2COC_6H_5$.

The change in thermal stability with increasing number of methylene groups between coordination sites for the copper chelates of a series of bis-(β-diketones) is shown in Table I.

TABLE I

THERMAL DECOMPOSITION TEMPERATURES OF COPPER CHELATES OF BIS(β-DIKETONES) OF THE TYPE $C_6H_5COCH_2CO(CH_2)_nCOCH_2COC_6H_5$

n	Decomposition by D.T.A.	Visual Decomposition
2	—	300 (darkens)
3	273	273 (darkens) 287 (melts)
4	272	279 (darkens) (does not melt)
5	258	272 (darkens) 278 (melts)
7	240	262 (melts and darkens)
8	230	231 (melts and darkens)

The temperature of the initial break in the base line of the differential plot was taken as the temperature at which the decomposition rate of the sample becomes appreciable. Although the peak maximum is a function of the heating rate, initial breaks are constant within the range investigated. Maxima would be expected to result from a complex relationship between the diffusion of heat into the sample and the heat of decomposition. A large dependence on heating rate would then be expected. Initial breaks in the differential plot are directly related to reactions within the sample. If heating rates are extremely slow, broad peaks are obtained and selection of the initial break is difficult. As differential thermal analysis equipment is more sensitive than is visual observation, it is not surprising that visual decomposition temperatures tend to be higher.

All decompositions are endothermic.

The linear decrease in thermal stability with increase in the methylene chain length is in line with the behavior of organic compounds containing extended chains. It indicates that simple cleavage of the metal ligand bond is not the mode of decomposition. If the complex site alone were cleaved, the decomposition temperature might be expected to be approximately constant.

The data indicate that no unusual thermal properties are conferred by the bis(β-diketone) coordination sites. The presence of the metal complex appears to present a mechanism which allows the hydrocarbon portion of the molecule to approach its true thermodynamic stability[3] more easily than in the case of the simple linear hydrocarbons.

EXPERIMENTAL

1,8-Diphenyloctane-1,3,6,8-tetraone (succinyldiacetophenone) was prepared by the method of Daub and Johnson,[4] m.p. 94–96°; reported 92–94°.

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[1] J. P. Wilkins and E. L. Wittbecker, U. S. Patent 2,659,711, November 17, 1953.

[2] E. H. Holst, Ph.D. dissertation, The Pennsylvania State University, 1958.

[3] G. S. Parks and H. M. Huffman, *The Free Energies of Some Organic Compounds*, Chemical Catalog Company, New York, N. Y., 1932, p. 99.

[4] G. H. Daub and W. S. Johnson, *J. Am. Chem. Soc.*, **72**, 501 (1950).