Ultraviolet, Visible, Circular Dichroism, and Electron Paramagnetic Resonance Spectra of the Copper(II) Complexes of Thyroxine and Thyroxine Analogs*

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ABSTRACT: The complexes of bis(L-thyroxinato)copper-(II) and its analogs have been studied in dimethyl sulfoxide solvent by spectroscopic methods. The structures that we find in this solvent are not in agreement with those presently in the literature for aqueous solvents. Since it seems likely that the structure should be similar in water and in dimethyl sulfoxide, we believe the previous structures to be unlikely. The present study has shown that the two carboxyl and the two amino functions are the complexing ligands. Iodination of the aromatic functions in these molecules changes the absorption spectrum relative to the noniodinated amino acid complexes, but these changes are consistent with a symmetry indicating gross tetragonal distortion of the complex rather than octahedral symmetry. Electron paramagnetic resonance measurements distinguish between a *cis*-ligand geometry as in bis(glycinato)copper(II) and a *trans*-ligand geometry as in bis-(thyroxinato)copper(II). A new formulation for the structure of bis(thyroxinato)copper(II) is presented.

The formation of complexes involving amino acids and cupric ion was reported more than 100 years ago (Gössmann, 1854), but the crystal structures of these complexes have only recently been investigated (Freeman *et al.*, 1964; Barclay and Stevens, 1963; Gramaccioli, 1963; Tomita and Nitta, 1961; Freeman, 1966). The reaction of thyroxine and its analogs with cupric ion has been studied in solution; however, the crystalline complexes have never been isolated, and the structures of these complexes are still unsolved. The investigations of Gemill (Gemill, 1951a,b, 1953), utilizing the ultraviolet absorption of thyroxine and related compounds, and of Frieden (Frieden and Naile, 1954; Frieden and Maggiolo, 1957), utilizing studies of chemical reactivity of bis(L-thyroxinato)copper(II), have resulted in their proposing structures I (Frieden, 1962; Wahlborg and Frieden, 1965) and IIa,b (Chart I) (Gemill, 1960; Litwack, 1964) for this complex. The stoichiometry of thyroxine and thyroxine-type analog copper(II) complexes in aqueous media has been established as being of the usual (amino acid)$_2$copper(II) type (Davis, 1957). Using the Job method of continuous variations (Job, 1928; Vosburgh and Cooper, 1941) we have established that the same stoichiometry holds for these complexes in dimethyl sulfoxide solvent. These results are presented graphically in Figure 1. The proposed structures are sufficiently different from the structures that have been previously found (Freeman, 1966) for complexes of the type bis(amino acid)-copper(II) that we have reinvestigated the problem using spectroscopic methods. The results of this investigation suggest that both hypothetical formulations are incorrect.

Experimental Methods and Procedures

Dimethyl sulfoxide (DMSO) (Baker Analyzed reagent grade), cupric perchlorate·6H_2O (G. F. Smith Co.), and amino acids (Sigma Chemical Co. and sources noted below) were used as obtained without further purification. 3,3′,5′-Triiodo-L-thyronine and 3,3′-diiodo-L-thyronine were provided by the Warner-Lambert Research Institute, Morris Plains, N. J. 3,5,3′-Triiodo-D-thyronine, 3,5-dibromo-3′,5′-diiodo-L-thyronine, 3′-isopropyl-3,5-diiodo-L-thyronine, 3′-ethyl-3,5-diiodo-L-thyronine, 3,5-diiodothyropropionic acid, 3,5-diiodothyroacetic acid, 3,5,3′-triiodothyropropionic acid, and 3,5,3′-triiodothyroacetic acid were provided by Smith Kline and French Laboratories, Philadelphia, Pa. L-Thyroxine, D-, L-, and DL-3,5,3′-triiodothyronine, and D-, L-, and DL-3,5-diiodothyronine were provided by Lederle Laboratories, Pearl River, N. Y. Solutions were prepared by dissolving the amino acid in the calculated volume of 1×10^{-2} M cupric perchlorate in DMSO to give a solution containing at least a two-fold excess of amino acid above the amount required to form the bis(amino acid)copper(II) complex.

Solutions for the Job analysis were prepared from a stock solution of 1×10^{-2} M sodium (L)-thyroxine pentahydrate (Sigma, lot no. 106B-0210) in DMSO and 1×10^{-2} M $Cu(ClO_4)_2 \cdot 6H_2O$ in DMSO. Solutions

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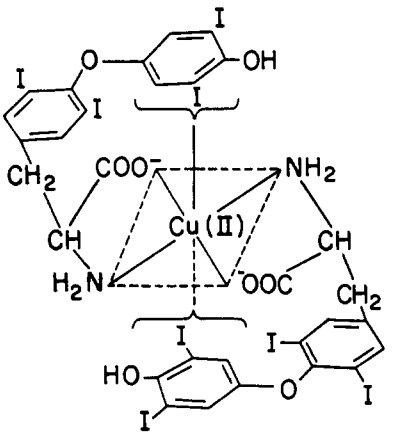
CHART I



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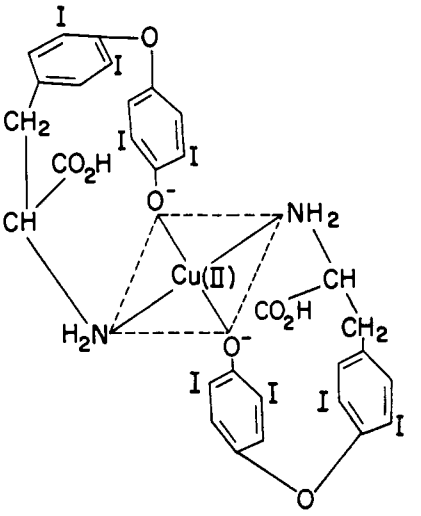
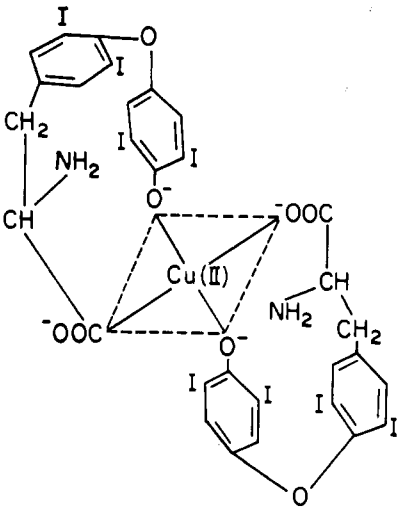


IIa



IIb

for spectral analysis were prepared by placing 0, 1, 2, 2.5, 3, 3.3, 4, 5, 6, 7, 8, and 9 ml of the copper solution in a 10-ml volumetric flask and then adding the solution of sodium thyroxine to the 10-ml mark. Spectra were scanned from 800 to ~480 mμ by using 4-cm light-path cells. The observed absorbance readings were then corrected for excess copper or excess thyroxine as required.

Ultraviolet and visible spectra were obtained with a Cary Model 15 spectrophotometer. Circular dichroism (CD) spectra were obtained with a JASCO Model 5 instrument. The cells used in the spectrophotometers were obtained from the Opticell Co. (Brentwood, Md.).

Electron paramagnetic resonance (epr) spectra were obtained with a Varian V4500 spectrometer using 100-kcycles/sec field modulation. Low-temperature spectra of the DMSO glasses were obtained using a variable temperature system utilizing a liquid nitrogen boil-off device as a source of cryogenic cooling. g values were determined by means of the Fieldial and a Hewlett-Packard X532B frequency counter. The stated accuracy of these components are such that the g values cannot be determined with an accuracy greater than 0.2%. We found that the g value of strong pitch could be determined to 0.001 (the range of values being 2.002–2.004 which compares well with the value of 2.0028 reported in the Varian User's Manual). However, in view of the above-stated instrumental limitation coupled with the inherent difficulty in assigning the resonance field corresponding to the g value in these fairly broad spectra, we take a conservative view of the accuracy of our g-value measurements.

Results

Ultraviolet and Visible Spectra. The ultraviolet spectra of thyroxine and some of its analogs were reported by Gemill (1955, 1956). These spectra were

obtained from aqueous solutions that were approximately 10^{-6} M in thyroxine and covered a wavelength span of 360–260 mμ. Solutions of thyroxine were reported to exhibit a single maximum that varied with pH. Gemill (1956) reported that in 0.04 N KOH thyroxine showed λ_{max} 325 mμ (ε 6207), and in 0.04 N HCl it showed λ_{max} 295 mμ (ε 4160). Figure 2 presents spectra of thyroxine and some of its analogs in DMSO solvent for the wavelength range 450–250 mμ. In this solvent thyroxine was found to exhibit λ_{max} at 355 mμ (ε 880), 300 (4573), and 265 (9598); $\lambda_{shoulder}$ 295 mμ (ε 4100). There is considerably more detail in this spectrum than had been reported previously.

Our observation that DMSO could be used to prepare concentrated solutions (*ca.* 10^{-2} M) of the cupric complexes of thyroxine and its analogs enabled us to overcome the difficulties previously encountered in obtaining spectra of these complexes in the visible region of the spectrum (Gemill, 1953). The spectrum of bis(L-thyroxinato)copper(II) exhibits λ_{max} 610 mμ (ε 82), 346 (222), and 330 (1550). Figure 3 presents the spectra of these bis(amino acid)copper(II) complexes from 800 to 320 mμ.

Circular Dichroism. Studies of circular dichroism of the bis(L-thyroxinato)copper(II) complex and of related cupric complexes revealed that the single rather broad absorption in the 600-mμ region was really a composite of several absorptions. The CD curves presented in Figure 4 show the presence of three bands for bis(L-thyroxinato)copper(II) at 700, 640 (sh), and 590 mμ. Furthermore, all of the complexes studied exhibit very similar curves.

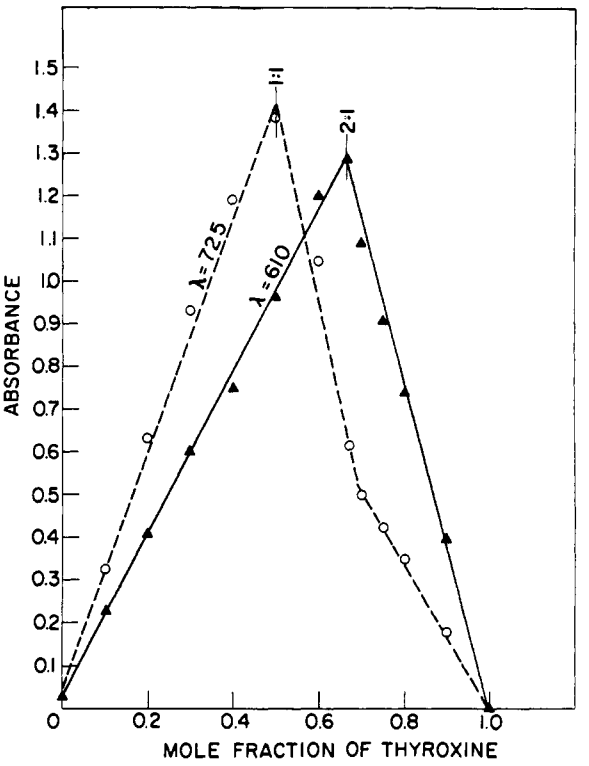


FIGURE 1: Job plot of $Cu(ClO_4)_2$ and sodium thyroxine in DMSO solvent.

TABLE I: Magnetic Parameters of Complexes.[a]

Bis(amino acid)copper(II)	$G_\perp$	$G_\parallel$	$A_\parallel$ (gauss)
Glycine	2.064	2.250	164
L-Alanine	2.054	2.232	176
L-Phenylalanine	2.053	2.233	176
Cyclopentane	2.053	2.230	176
L-Thyroxine	2.053	2.236	176
L-Thyronine	2.051	2.235	176
3,5,3′-Triiodo-D-thyronine	2.053	2.232	176
3,5-Dibromo-3′,5′-diiodo-L-thyronine	2.056	2.243	176
3′-Isopropyl-3,5-diiodo-L-thyronine	2.053	2.242	176
3′-Ethyl-3,5-diiodo-L-thyronine	2.051	2.232	176
$Cu(ClO_4)_2$	2.081	2.381	112
3,5-Diiodothyropropionic acid	2.083	2.389	112
3,5,3′-Triiodothyropropionic acid	2.076	2.394	108
3,5-Diiodothyroacetic acid	2.082	2.382	108
3,5,3′-Triiodothyroacetic acid	2.067	2.394	108

[a] Solvent, DMSO; field modulation, 1.5 gauss; 1×10^{-3} M copper(II) perchlorate; temperature, −165°; power, 2.7 mw; and 1×10^{-2} M amino acid.

Epr. The results of the epr studies are tabulated in Table I. Typical spectra of bis(glycinato)copper(II), bis(L-thyroxinato)copper(II), and uncomplexed Cu(II) are presented in Figure 5. The differences between the glycine complex and the complexes of Cu(II) with alanine and thyroxine have been carefully checked and are real. The other amino acid cupric complexes investigated are listed in Table I. Their epr spectra were identical with the spectrum presented in Figure 5 for bis(L-thyroxinato)copper(II). Acids that did not complex or interacted weakly gave epr spectra identical with the spectrum presented in Figure 5 for $Cu(ClO_4)_2$.

Model Building. In order to study the spatial relationships of the atoms in bis(L-thyroxinato)copper(II) attempts were made to construct molecular models of structures I and IIa,b. Using CPK[1] models in which a generalized metal atom having the following dimensions was utilized: covalent radius 1.32 A, van der Waals radius 1.70 A, and bond angle $\alpha = \beta = \gamma = \Delta$

[1] Abbreviation used: CPK, Corey, Pauling, and Koltun molecular models.

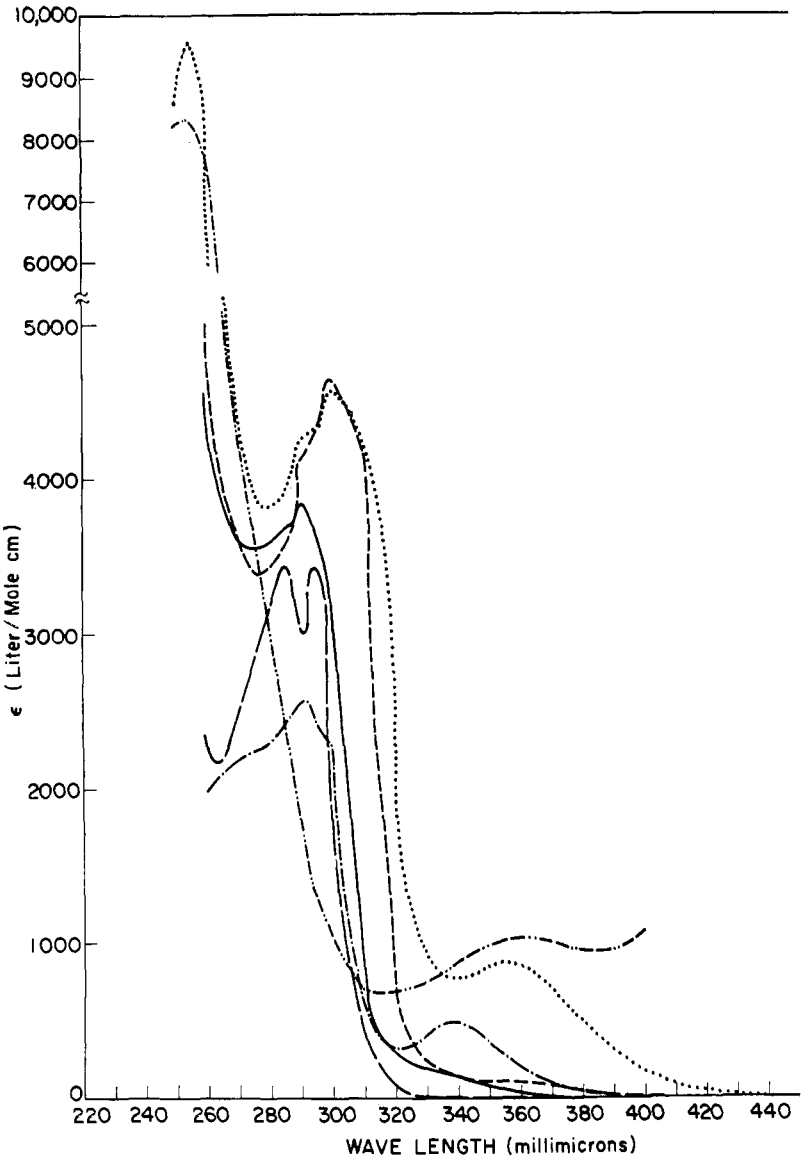


FIGURE 2: Ultraviolet spectra of thyroxine and some analogs. (· · · ·), L-thyroxine; (— —), L-thyronine; (– – – –) 3,5,3′-triiodo-L-thyronine; (———), 3,5-diiodo-L-thyronine; (— · —), 3,5-diiodo-L-tyrosine; and (– – · ·), 3,5-dinitro-L-tyrosine.

= 90°; structure I could only be made if the amino and carboxyl ligands were in a *cis* relationship when attached to the copper atom and structures IIa and b could not be constructed.

The failure to construct models of structure IIa,b does not in itself preclude that one of these structures may in fact be correct. In the models that were constructed, the presence of interactions between the three and five iodine atoms of one ring and the 2′- and 6′-hydrogen atoms of the second ring was observed. This observation is contrary to the report by Gemill (1960) and Litwack (1964) that thyroxine exhibits considerable freedom of rotation between the diphenyl ether rings.

Discussion

Discussions of the visible spectra of cupric complexes have appeared recently in papers by Brill *et al.* (1964), Koltun *et al.* (1963), and Freeman (1966). The spectra that we have obtained for the cupric complexes of thyroxine and its analogs may be divided into two groups. The complexes with iodinated amino acids exhibit λ_{max} 610 mμ (ε 82) and 410–330 mμ (ε 15–1500), whereas the complexes with noniodinated amino acids have λ_{max} in the region 660–640 mμ (ε 50) and exhibit no absorption in the range 410–330 mμ. The fact that the spectra of bis(3,5-diiodotyrosine)-copper(II) and bis(thyroxinato)copper(II) are so similar

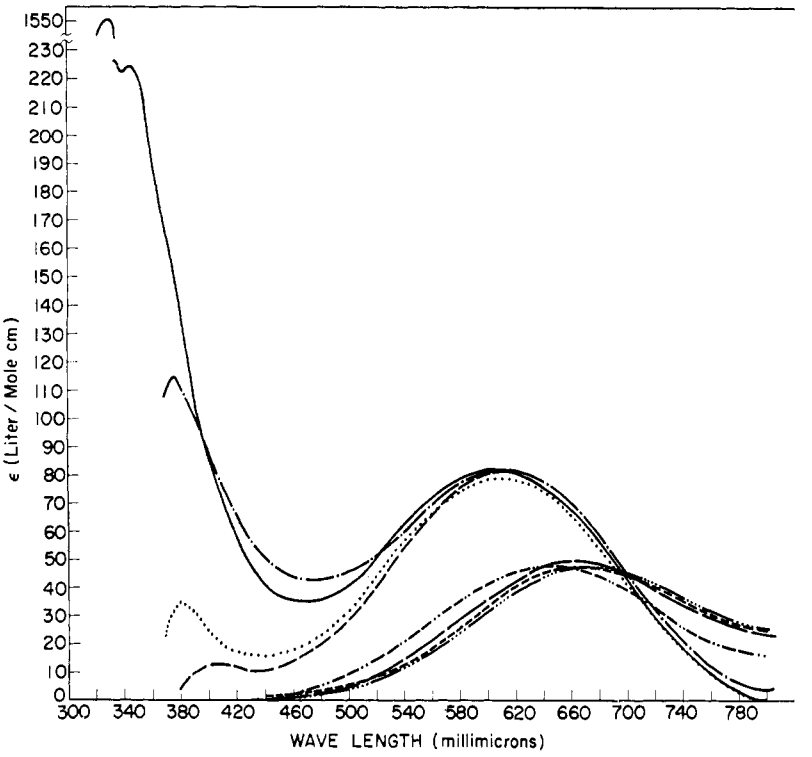


FIGURE 3: Visible spectra of bis(L-thyroxinato)copper(II) and some analogs. (—···—), tyrosine; (- - - -), thyronine; (– – · ·), alanine; (— —), phenylalanine; (— · —), 3,5-diiodothyronine; (· · · ·), 3,5,3′-triiodothyronine; (———), thyroxine; and (– – – –), 3,5-diiodotyrosine.

in intensity and shape at 610 mμ implies that it is not the substituents on the prime ring that are responsible for the change in intensity and absorption maximum. If structure I were in fact correct, we would expect to see differences in the spectra of bis(3,5-diiodotyrosinato)copper(II) and bis(thyroxinato)copper(II) in the region of 610 mμ. The fact that such differences are not observed indicates that the formulation is probably incorrect. Both Brill *et al.* (1964) and Freeman (1966) have proposed approximations based on the visible absorptions of cupric complexes that permit correlation of the nature of the ligand atoms in these complexes with their absorption maxima. Thus $\lambda_{max} > 700$ mμ indicates predominantly oxygen ligands, $\lambda_{max} \approx 630$ mμ indicates two nitrogen ligands, and $\lambda_{max} < 550$ mμ indicates predominantly nitrogen ligands. The spectra of cupric complexes of a number of α-amino acids were reported by Li and Doody (1954). They reported that the formation of a cupric–α-amino acid (1:1) complex was characterized by an absorption maximum in the region of 700 mμ, whereas formation of the 2:1 bis complex was characterized by an absorption maximum in the region 650–615 mμ. This is in agreement with the data presented in Figure 1. According to Brill *et al.* (1964) the shape of the absorption curves shown in Figure 3 is indicative of a gross tetragonal distortion of the four nearest neighbors surrounding the copper atom. If the λ_{max} and ε values obtained for the bis(amino acid)copper(II) complexes that were examined in this study are correlated with the approximations proposed for coordination number and ligand-atom type, it would seem that under the experimental conditions employed the 2:1 complex utilizing two nitrogen and two oxygen atoms (amino and carboxyl) is the most likely species present. The present analysis assumes that the structure of these complexes in DMSO will not be very different from the structure in water solvent. This assumption is based upon the fact that the DMSO was not anhydrous and upon the similarity of the spectra of the complexes in the region of 700–550 mμ to spectra reported for aqueous solutions of other bis(amino acid)copper(II) complexes.

The factors determining the solid state and solution geometry of bis(amino acid)copper(II) complexes appear to be complex and controversial. Until very recently all the bis(amino acid)copper(II) complexes, except bis(glycinato)copper(II), studied by X-ray methods were found to have *trans*-ligand geometries (Freeman, 1966). Bis(glycinato)copper(II) was the exception and was assigned a *cis*-ligand configuration (Freeman *et al.*, 1964; Tomita and Nitta, 1961). The structure of bis(alaninato)copper(II) was determined by Dijkstra (1966) and was found to have a *trans* configuration. Gillard *et al.* (1966) have shown that the *trans* compound could be converted to the *cis* isomer by prolonged equilibration of crystals of the

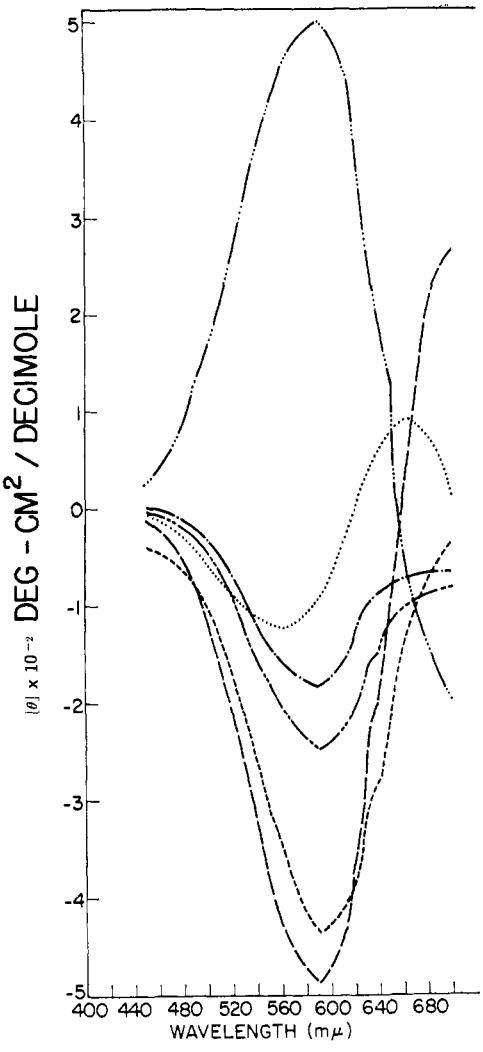


FIGURE 4: CD spectra of bis(L-thyroxinato)copper(II) and some analogs. (— —), L-thyroxine; (- - - -), L-phenylalanine; (— · —), L-thyronine; (— - -), L-thyroxine; (— · ·), 3,5,3′-triiodo-D-thyronine; and (· · · ·), 3,5-diiodo-L-thyronine.

trans complex in water. These workers suggest that the crystalline complexes of α-amino acids with Cu(II) have configurations determined in large part by kinetic factors rather than thermodynamic factors. It has also been suggested that steric factors may exert considerable influence in determining the geometry of bis(amino acid)copper(II) complexes (Wellman *et al.*, 1966). The data from the above studies can be best interpreted by the assumption that in solution the configuration of the bis(amino acid)copper(II) complexes is predominantly the *trans* species, and that this conformation has considerable stability relative to the *cis* isomer.

Thyroxine and its analogs may be considered grossly as alanine derivatives and the cupric complexes of alanine and thyroxine might, therefore, be expected to have a stereochemically similar ligand configuration in solution. Additional information on the arrangement of ligands in the bis(L-thyroxinato)copper(II) complex was sought from CD and epr studies. Yasui *et al.* (1965) have studied the CD of several copper–amino acid complexes in aqueous solution. They observed a small positive band at 730 mμ and two negative bands at 630 and 565 mμ for bis(L-alaninato)copper-(II). In the present studies the bis(L-thyroxinato)copper-(II) complex showed a positive band a little above 700 mμ and two negative bands at 640 and 590 mμ. These shifts are not unexpected in view of the shifts observed in the absorption spectra of these complexes.

The absorption spectra of some bis(α-amino acid)-copper(II) complexes in solution and in single crystals have been analyzed by Dijkgraaf (1964). A tentative assignment of transition levels for such complexes is made by Dijkgraaf in his analysis. However, unconditional assignments are not feasible at this time because of the difficulty in predicting transition levels when the symmetry of Cu(II) complexes is reduced from square-planar (D_{4h}) to a tetragonally distorted symmetry (crudely C_{2h}).

Comparison of the absorption and CD spectra obtained for bis(L-thyroxinato)copper(II) and its analogs with the absorption and CD spectra of bis(L-alaninato)-copper(II), bis(L-phenylalaninato)copper(II), and bis-(L-tyrosinato)copper(II) reveals so much similarity that we believe that the ligand atoms and their approximate configurations are roughly the same for all of these complexes.

The results of the epr measurements at both X band (9 kMHz) and K band (35 kMHz) may be viewed as providing corroborative evidence for the predominance of a *trans*-ligand configuration in the bis(L-thyroxinato)copper(II) complexes. The complexes can be placed into three categories distinguished by their g values. These categories are exemplified by: (1) bis(glycinato)copper(II) (this is the only member in this category); (2) bis(L-alaninato)copper(II) and all other copper amino acids studied in this investigation; and (3) copper perchlorate and other weakly complexed structures such as the cupric thyroacetic and thyropropionic acids. The outstanding feature of the data is that bis(glycinato)copper(II) can be distinguished from the other copper–amino acid complexes on the basis of its epr spectrum. This complex is known to have a preferred *cis* configuration of ligands.

The observed epr line shape merits special comment. Qualitatively the epr spectra resemble that expected for a spin one-half system in a rhombic crystal field, one of the resonances (g_z) being further split by virtue of the electron–nuclear hyperfine interaction. However, this interpretation does not bear up under closer scrutiny. For instance, according to second-order perturbation theory, the high-field resonance, if it were a true g value (*i.e.*, g_x), would arise from a large splitting in the excited states in the visible region for which there is no evidence. We have, therefore, obtained the epr spectra for some of these complexes at higher frequency (Figure 5b). At the higher frequency we find the spectra

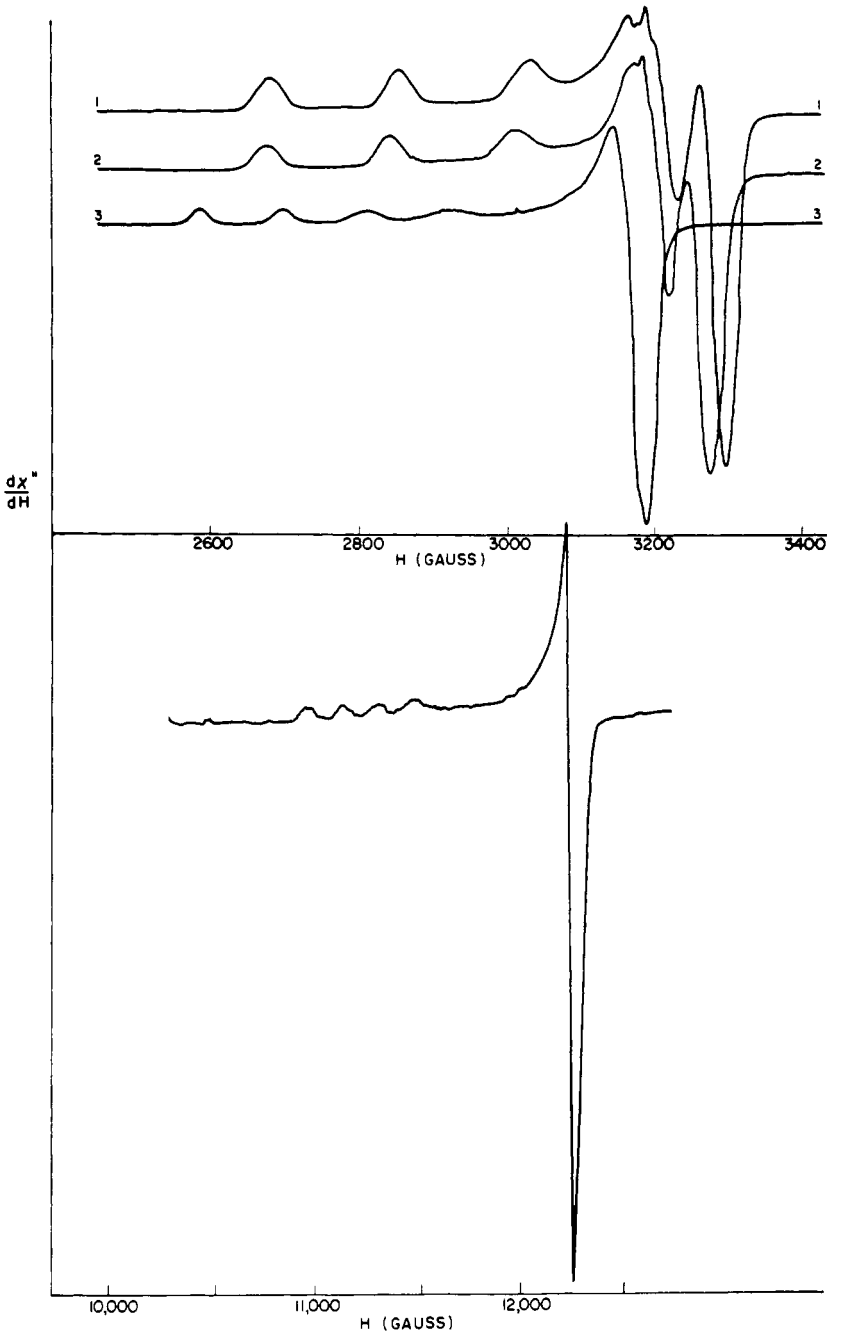
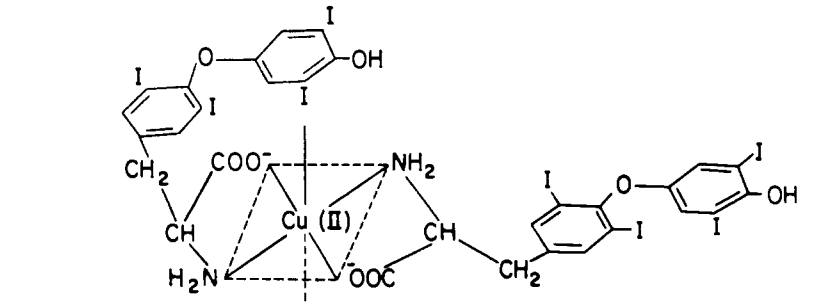


FIGURE 5: Epr spectra of Cu(II) complexes. (a) (top) X-band spectra of: (1) bis(L-thyroxinato)copper(II), (2) bis-(glycinato)copper(II), and (3) copper perchlorate. (b) (bottom) K-band spectrum of bis(L-thyroxinato)copper(II).

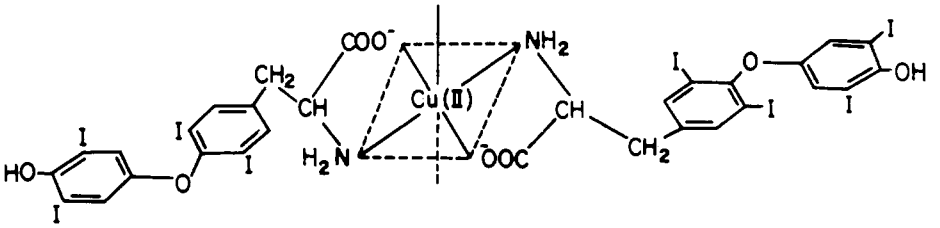
to exhibit a simple axial line shape. This confirms the assumption that the high-field resonance is not a true g value. The explanation of this anomaly is to be found in the work of Neiman and Kivelson (1961) who have shown that when the magnitude (in gauss) of the hyperfine interaction approaches the separation of g values, it is possible for accidental turning points in the angular distribution to occur.

The chemical behavior of the cupric complexes of thyroxine and its analogs has led to suggestions that these complexes possess greater stability constants than other cupric–amino acid complexes. The stability constants (K_s) for bis(L-alaninato)copper(II), bis(L-phenylalaninato)copper(II) (Albert, 1950), and bis(L-trysosinato)copper(II) (Albert, 1952) have been reported as $10^{15.1}$, $10^{14.9}$, and $10^{15.0}$, respectively. Only when the amino acid possesses an unusual structural feature that affects the ligand field do the stability constants increase or decrease appreciably. Thus, reported K_s values for bis(histaminato)copper(II) of

CHART II



III



IV

$10^{16.2}$, bis(ethylenediamine)copper(II) of 10^{20} (Albert, 1952), and bis(prolinato)copper(II) of $10^{16.8}$ (Albert, 1950) reflect this ligand-field effect. Although a value of 10^{12} has been suggested for bis(L-thyroxinato)-copper(II) (Wahlborg and Frieden, 1965), the spectral evidence obtained in this study suggests that the K_s of bis(L-thyroxinato)copper(II) and its analogs ought to be closer to 10^{15}. It has been suggested that increased stability in bis(L-thyroxinato)copper(II) could arise *via* axial interactions, such as shown in structure I. This structure, however, requires that copper have a coordination number of six and this is unlikely in bis-(amino acid)copper(II) complexes. A coordination number of six occurs very rarely for cupric complexes in solution. Even in the solid state a more usual coordination number for cupric complexes is four or five (Freeman, 1966). At present there is no evidence for any such enhanced stability in bis(L-thyroxinato)-copper(II) complexes.

The information obtained from this study coupled with the literature data on Cu(II) complexes of amino acids suggest that the structure of bis(L-thyroxinato)-copper(II) could best be described by structures III or IV (Chart II). These structures are compatible with all available ultraviolet, visible, CD, and epr spectra. Structure I is considered to be incorrect because the spectra are not compatible with an octahedral symmetry and because it requires that the ligands have a *cis* configuration when complexed with Cu(II). Structures IIa and b are considered to be incorrect because they require too great a distortion of the bond angles to be formed, and all of the present spectral evidence is best interpreted as indicating that the ligands are two oxygen atoms from carboxyl groups and two nitrogen atoms from the amino groups. We can find no evidence to support bonding to copper *via* the hydroxyl function and the carboxyl or amino functions as shown in structures IIa and b.

Acknowledgment

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