Nature of Copper(II) Interaction with Thyroxine and Analogs[1a]

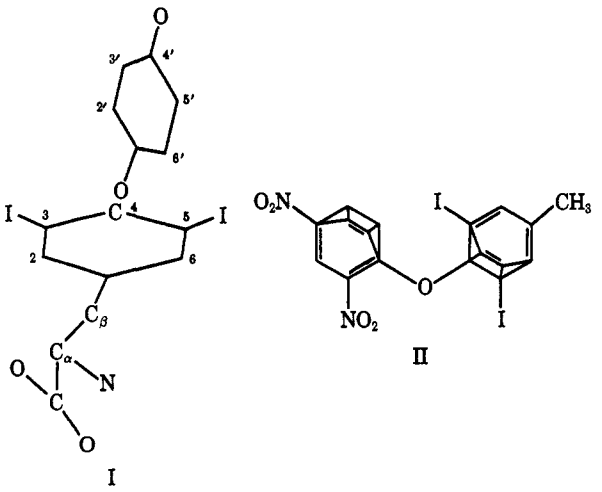
W. R. Walker,[1b] Yueh-Ho L. Shaw,[1c] and Norman C. Li*

Contribution from the Department of Chemistry, Duquesne University, Pittsburgh, Pennsylvania 15219. *Received October 25, 1972*

Abstract: The interaction of thyroxine and its analogs, 3,5,3′-triiodothyronine, 3,5-diiodothyronine, and 3,5,-diiodotyrosine, with copper(II) has been investigated by nmr spectroscopy in dimethyl sulfoxide and in D_2O. Selective line-broadening studies of the nmr signals by traces of paramagnetic copper(II) have been carried out. Some evidence for a copper(II)–iodine interaction has been obtained. This confirms an earlier proposal by Zand and Palmer that the copper assumes 5-coordination by axial interaction with iodine at the apex of a square pyramid.

In recent years, thyroid hormones have received particular attention especially with regard to the relationship between their physiological function and their structure. Cody, *et al.*,[2] have for the first time reported the structure of a compound containing the thyroxine nucleus. This is a 1:1 complex of 3,5-diiodo-L-thyronine with *N*-methylacetamide, and an end-on view of this thyroxine analog is shown in I.

This symmetrically twisted conformation is similar to that of 2,6-diiodo-4-methylphenyl 2′,4′-dinitrophenyl ether (II) which has been investigated by Lehman.[3] He suggested that, in this compound, one iodine atom is positioned so that its orbitals overlap extensively with the densest part of the π cloud of the other ring. He further proposes that such a charge-transfer complex makes thyromimetric activity possible and explains the known structure–activity relationship of the thyroid hormones and their synthetic analogs.

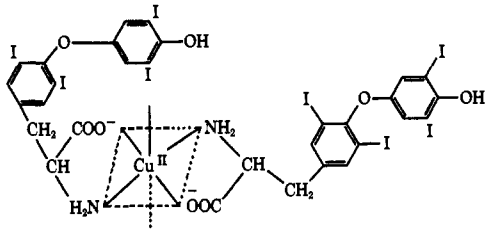


I

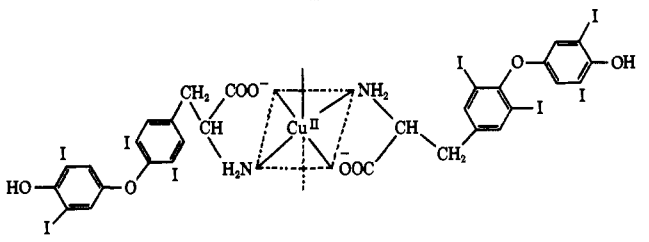
II

Another recent report[4] has stated that thyroxine is converted into triiodothyronine by isolated perfused rat heart. The latter compound is supposedly four to five times as potent as thyroxine in causing tachycardia in some patients and has indeed been isolated from human plasma.

The complexes of bis(L-thyroxinato)copper(II) and its analogs have been studied by Zand and Palmer[5] in dimethyl sulfoxide (DMSO) by a variety of spectroscopic methods. Such methods included ultraviolet and visible spectra, circular dichroism, and electron spin resonance studies. From the data obtained they concluded that the structure of copper(L-thyroxine)$_2$ could best be described as being III or IV.



III



IV

Because of interest in the interaction of bivalent copper with other amino acids and biologically important compounds, the nuclear magnetic resonance (nmr) spectra and electronic spectra of the copper(II) thyroxine system and other thyroxine analogs have been investigated in DMSO and D_2O.

Experimental Section

Materials. DMSO (Fisher Scientific) was redistilled and dried over Davison molecular sieves (Fisher Scientific). Fully deuterated DMSO and D_2O were supplied by Diaprep, Inc. Water was twice distilled. The following amino acids were used as supplied: L-thyroxine (sodium) pentahydrate, 3,5,3′-triiodo-L-thyronine, and DL-tyrosine (Nutritional Biochemicals Corp.), 3,5-diiodo-L-thyronine and 3,5-diiodo-L-tyrosine (Kurt J. Lesker Co., Pittsburgh, Pa.). The compounds 3,5-dimethyltyrosine and 4-methoxy-3,5-dimethyltyrosine are gifts of Dr. C. K. Shaw.

Method. Nmr spectra were obtained with a Varian A-60 nmr spectrometer at 60 MHz and at an ambient temperature of 33 ± 1°. Care was taken to keep the radiofrequency power level well below

(1) This investigation was supported by National Science Foundation Grant No. GB-25117. (b) Department of Chemistry, The University of Newcastle, Newcastle, N. S. W., Australia; (c) Yale University Medical School, New Haven, Conn.

(2) V. Cody, W. L. Duax, and D. A. Norton, *Chem. Commun.*, 683 (1971).

(3) P. A. Lehman, Abstracts, XXIII IUPAC Conference, Boston, Mass., 1971, p 70.

(4) J. L. Rabinowitz and E. S. Hercker, *Science*, **173**, 1242 (1971).

(5) R. Zand and G. Palmer, *Biochemistry*, **6**, 999 (1967).

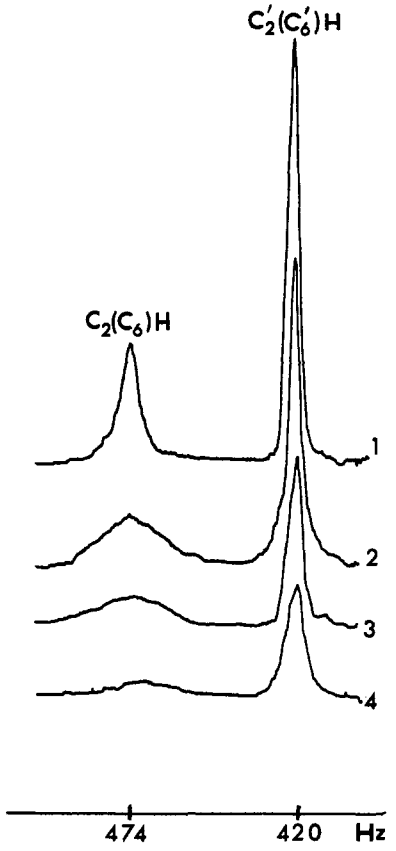


Figure 1. Nmr spectra of 0.3 M thyroxine in DMSO containing different concentrations of $CuCl_2$: (1) 0; (2) 2×10^{-4} M; (3) 6×10^{-4} M; (4) 8×10^{-4} M.

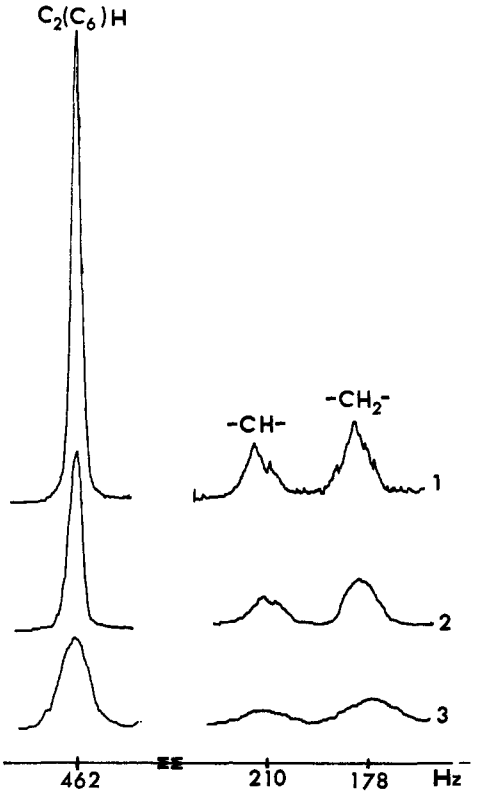


Figure 2. Nmr spectra of 0.3 M 3,5-diiodotyrosine in DMSO containing different concentrations of $CuCl_2$: (1) 0; (2) 4×10^{-4} M; (3) 2×10^{-3} M.

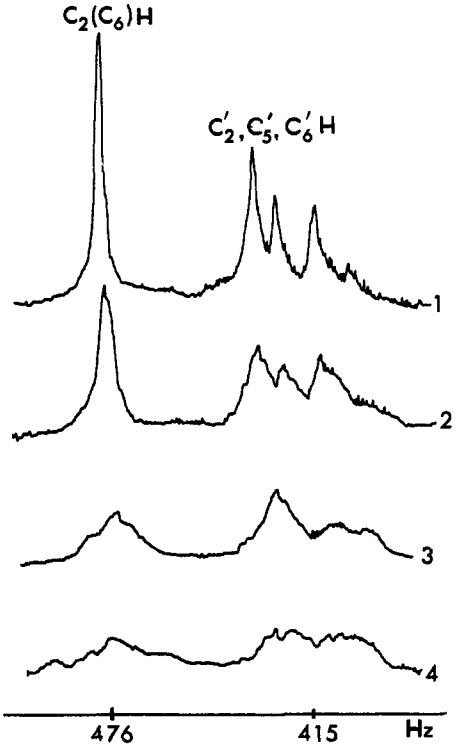


Figure 3. Nmr spectra of 0.3 M 3,5,3′-triiodothyronine in DMSO containing different concentrations of $CuCl_2$: (1) 0; (2) 4×10^{-4} M; (3) 2×10^{-3} M; (4) 4×10^{-3} M.

saturation and the field homogeneity such that a resolution of 0.3 Hz or better was attained. The chemical shifts of the important signals were measured with respect to 1% tetramethylsilane (TMS).

Optical spectra were recorded with a Cary 14 recording spectrophotometer at room temperature. Quartz cells of 1, 5, and 10 cm were used depending on the solution concentrations.

Measurements of pD were made with a Corning Model 10 pH meter with external electrodes. Only freshly prepared solutions were used.

Results and Discussion

The nmr spectrum of 0.3 M L-thyroxine (sodium) pentahydrate was recorded in DMSO with TMS as an internal reference. As may be seen in Figure 1, the signals of the two pairs of equivalent C–H protons appear at 420 and 474 Hz and are assigned to 2′,6′ and 2,6 protons, respectively. This agrees with an earlier assignment.[6] When anhydrous $CuCl_2$ was added in increasing amounts, both signals were selectively broadened by the paramagnetic copper ion; the 2,6 protons, however, were affected much more than the 2′,6′ protons. This is strong evidence for the iodine atoms on C_3 or C_5 interacting with Cu^{2+} or that the iodine atoms on C_3' or C_5', as shown in III, are interacting.

Further evidence for this copper(II)–iodine interaction appears in Figure 2 where the spectrum of 0.3 M 3,5-diiodo-L-tryosine in DMSO is shown. Here the signals of $-CH_2-$, –CH–, and the 2,6 protons appear at 178, 210, and 462 Hz, respectively. On the addition of $CuCl_2$, the –CH–, $-CH_2-$, and the 2,6 proton signals are all broadened.

A similar result was obtained by adding $CuCl_2$ to 0.3 M 3,5,3′-triiodothyronine in DMSO. The spectrum (Figure 3) shows the two equivalent 2,6 protons

(6) P. A. Lehman and E. C. Jorgenson, *Tetrahedron*, 21, 363 (1965).

at 474 Hz (the same as in thyroxine) and the 2′,6′, and 5′ protons as a multiplet centered around 415 Hz. The addition of $CuCl_2$ results in a broadening of all signals and although the 2,6 signals were most affected, this may not be due to the Cu^{2+}–iodine interaction as much as the proximity of C_2H and C_6H to the "glycine-like" bonding site, which is the amino nitrogen and the carboxyl oxygen.

Table I gives the half-width in Hz of nmr proton signals for tyrosine and its analogs (0.2 *M* in D_2O, pD ~12), as a function of $CuCl_2$ concentration. It is seen that the broadening effect of copper(II) on the C_2–(C_2–(C_6)H and on the C_3–(C_5)CH_3 signals is greater for 3,5-dimethyltyrosine that for 4-methoxy-3,5-dimethyltyrosine. This can only be explained if there is some interaction between the oxygen atom on C_4 and the Cu^{2+} ion. The large broadening effect of Cu^{2+} ion on the C_2–(C_6)H of 3,5-diiodotyrosine, compared with the C_2–(C_6)H of tyrosine analogs which contain no iodine atom, can be explained by the interaction between iodine and the copper(II) ion.

Table I. Half-Width of Proton Signals for Tyrosine and Its Analogs (0.2 *M* in D_2O, pD ~12), as Function of $CuCl_2$ Concentration

		—[$CuCl_2$] × 10^4, *M*—			
		0	1.0	3.0	5.0
	Group	Half-width, Hz			
3,5-Diiodotyrosine	C_2–(C_6)H	1.5	2.4	4.6	7.0
3,5-Dimethyltyrosine	C_2–(C_6)H	2.6	3.0	3.6	5.5
4-Methoxy-3,5-dimethyltyrosine	C_2–(C_6)H	2.8	3.2	3.5	4.6
3,5-Dimethyltyrosine	CH_3	2.0	2.1	2.9	3.8
4-Methoxy-3,5-dimethyltyrosine	CH_3	1.7	1.9	2.0	2.6
4-Methoxy-3,5-dimethyltyrosine	OCH_3	1.2	1.3	1.5	1.6

Zand and Palmer[4] have shown that copper(II) complexes of iodinated amino acids in DMSO have λ_{max} at 610 nm (ϵ ~80) and also possess a charge-transfer band between 330–410 nm (ϵ 15–1500). Noniodinated amino acids exhibit no absorption in the near uv because the charge-transfer band is missing. Copper(II) complexes of the three iodinated amino acids, thyroxine, 3,5-diiodothryonine and 3,5,3′-triiodothyronine, all possess similar spectra. Our spectral data in Table II agree with those of Zand and Palmer.

Table II. Electronic Spectral Data for DMSO Solutions Containing 0.02 *M* $Cu(ClO_4)_2 \cdot 6H_2O$

			Charge-transfer band	
0.1 *M*	λ_{max}, nm	ϵ	λ_{max}, nm	ϵ
Thyroxine	610	77	330	1550
3,5-Diiodothyronine	610	80	380	115
3,5,3′-Triiodothyronine	610	80	380	35
3,5-Diiodotyrosine	610	77	410	14

If the intensity of the charge-transfer band can be taken as a criterion of the copper–iodine interaction, it would appear that this is a maximum in thyroxine and a minimum in 3,5-diiodotyrosine. This strongly indicates that structure III, as postulated by Zand and Palmer,[4] is the most probable.

The claim[4] that the stoichiometry of copper(II)–thyroxine complexes is the same in DMSO as in water is supported by data from Laurie.[7] He showed that isomeric copper(II)–tyrosine complexes all gave identical spectra in water, pyridine, DMSO, and *N*,*N*-dimethylformamides.

It appears from the present study that nmr selective line broadening of signals by paramagnetic copper(II) and electronic spectral data do afford evidence for some sort of interaction between an iodine atom of thyroxine and analogs, and the copper to which these molecules are bound. It is suggested that the iodine atom interacts with the square-planar copper(II) ion that assumes square-pyramidal 5-coordination.

(7) S. H. Laurie, *Aust. J. Chem.*, **20**, 2615 (1967).

Communications to the Editor

Unsubstituted Cyclopentadienyl Cation, a Ground-State Triplet

Sir:

There has recently been considerable interest in such antiaromatic 4π-electron species as the cyclopropenyl anion, cyclobutadiene, and the cyclopentadienyl cation. Attention has focused not only on the energy of these species, but also on their electronic and geometric structures. In particular, these substances and any derivatives of them which retain their *n*-fold symmetry are predicted to be possible ground-state triplet, rather than normal singlet, molecules. In the case of cyclopentadienyl cation, our PPP–SCF calculations[1] indicate that the symmetrical triplet should be 0.318 eV more stable than the completely symmetrical singlet state. Of course, this 7.3 kcal/mol energy advantage must be balanced against the stabilization that the symmetrical singlet could gain by undergoing Jahn–Teller distortion, either to a somewhat less symmetrical planar structure[2] or perhaps even to more extreme geometries.[3]

A certain amount of evidence about this system is available. Thus, pentaphenylcyclopentadienyl cation[4,5] is a ground-state singlet whose triplet state lies approximately 1 kcal/mol higher. A number of other pentaarylcyclopentadienyl cations have also been

(1) Using standard values for integrals and all singly excited configurations. The S–T gap without configuration interaction is calculated to be 0.470 eV.

(2) L. Snyder (*J. Phys. Chem.*, **66**, 2299 (1962)) predicts that Jahn–Teller distortion to C_{2v} symmetry would stabilize the singlet by 5.25 kcal/mol.

(3) W.-D. Stohrer and R. Hoffmann, *J. Amer. Chem. Soc.*, **94**, 1661 (1972).

(4) R. Breslow, H. W. Chang, and W. A. Yager, *ibid.*, **85**, 2033 (1963).

(5) R. Breslow, H. W. Chang, R. Hill, and E. Wasserman, *ibid.*, **89**, 1112 (1967). The conversion factor on p 1114 should be 0.09348 cm^{-1} = 1000 G.