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Distal Conformation of Thyroid Hormones. Crystal and Molecular Structure of 3,5,3'-Triiodo-L-thyronine Methyl Ester

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In the crystal structure of 3,5,3'-triiodo-L-thyronine methyl ester, the 3'-iodine is distal, *i.e.*, away from the alanine bearing ring, and the overall conformation is cisoid, that is, the alanine moiety and the outer phenyl ring lie on the same side of the inner phenyl ring plane. This conformation, reported here for the first time, is in contrast to the transoid conformation previously observed for thyroid hormone structures. The torsional angles between the diphenyl ether linkages (ϕ and ϕ') are -108 and 33° , respectively, while the C-O-C angle is 117° . The value of χ^1 , which describes the amino acid backbone conformation, is 308° . The structure crystallizes in the tetragonal space group $P4_1$ with $a = 8.225$ (5) and $c = 28.42$ (1) Å. The final R index is 0.06.

Recently extensive studies of the molecular conformation of thyroid hormones and their analogs have been made in an effort to understand the structural requirements for their biological activity. There is now an accumulation of evidence which suggests that the hormone 3,5,3'-triiodo-L-thyronine (T_3) plays a more significant role in biological activity than had been previously supposed. Recent studies have also established that thyroxine (T_4) is converted to T_3 in peripheral tissues.¹⁻⁴ There are two conformers which can result from the deiodination of thyroxine (Figure 1), *i.e.*, a distal or proximal conformer depending upon whether the 3'-iodine is away from or toward the inner phenyl ring. The results of several stereochemical, binding, and biological activity studies⁵⁻⁸ strongly suggest that hormonal activity is greater for the distal orientation of the 3'-iodine.

Based on steric considerations, it had been suggested that a large barrier to internal rotation about the diphenyl ether linkage prevented easy conversion from one conformer to the other. However, recent energy calculations using modified CNDO/2 procedures⁹ and those using the extended Hückel approach¹⁰ both suggested that the energy barrier to internal rotation about the diphenyl ether linkage is relatively small (11 and 50 kcal, respectively). Neither study showed any significant preference for the distal or proximal conformer. The repeated crystallographic observation of both the distal^{11,12} and proximal^{13,14} 3'-iodine in triiodothyronines suggests that the two conformers are indeed stable and readily accessible in solution. We report here the first complete structural details of a thyroid hormone analog having an overall cisoid conformation with a distal 3'-iodine in the crystal and molecular structure of 3,5,3'-triiodo-L-thyronine methyl ester. This work is part of a larger program to study the

molecular conformation of a series of thyroid hormones, hormone precursors, and thyroxine analogs.

Experimental Section

Crystals of 3,5,3'-triiodo-L-thyronine methyl ester were prepared by methylation of T_3 and then removal of the HCl by extraction through a column of Fisher Rexyn 203(OH). Crystals were grown from methanol solutions. A clear, well-formed crystal ($0.10 \times 0.18 \times 0.20$ mm³) was selected for intensity data collection. All X-ray measurements were made on a GE-XRD-5 single crystal counter diffractometer. The crystal was mounted with the b axis parallel to the ϕ axis of the instrument. The systematic extinctions indicated the tetragonal space group $P4_1$. The unit cell dimensions were obtained from a least-squares calculation based on 2θ measurements of 34 hkl reflections having $2\theta > 40^\circ$, using the Mo $K\alpha$ radiation for data collection. The crystal data are presented in Table I.

The intensities of 1758 (1474 observed) independent reflections with 2θ less than 50° were measured by the stationary crystal-stationary counter technique. The background was a uniform function of 2θ above 20° and a background correction curve was constructed from zirconium-yttrium balanced filter measurements of the data in this range. Reflections were considered unobserved if the net count was less than twice the standard deviation of the background. Yttrium filtered background measurements were made for all data with 2θ less than 20° . No significant changes were observed in the intensities of the standard reflections measured daily during data collection. The shape anisotropy measurements at $\chi = 90^\circ$ were found to be less than $\pm 3\%$ over the ϕ range covered in the intensity collection. Intensities were corrected for Lorentz and polarization factors, and an absorption correction based on the intensity variation of ϕ at $\chi = 90^\circ$ was applied.

The structure was solved by straightforward application of heavy atom techniques. Atomic scattering factors for iodine were taken from Cromer¹⁵ while those for the other atoms were taken from the International Tables for X-Ray Crystallography.¹⁶ The positional parameters and anisotropic thermal parameters for all nonhydrogen atoms were refined by using a block diagonal ap-

Table I. Crystal Data for 3,5,3'-Triiodo-L-thyronine Methyl Ester

Mol formula	C ₁₆ H ₁₄ O ₄ NI ₃
Mol wt	664.96
Crystal system	Tetragonal
Space group	P4 ₁
Z	4
Cell dimensions, Å	a = 8.225 (5) c = 28.42 (1)
Cell volume, Å ³	1923.1
Calcd density, g/cm ³	2.29
F(000)	308
Crystal size, mm	0.10 × 0.18 × 0.20
Final R, %	13.0 (1693) all data for which F _c / F _o > 0.5 6.1 (1535) observed data for which F _c / F _o > 0.5

Table II. Positional Parameters for 3,5,3'-Triiodo-L-thyronine Methyl Ester

	X/A	Y/B	Z/C
I3	0.1429 (1)	0.1536 (1)	0.7040 (0)
I5	0.4729 (1)	0.7766 (1)	0.6425 (0)
I3'	0.1978 (1)	0.9653 (1)	0.8401 (0)
C1	0.2928 (25)	0.3420 (22)	0.5696 (6)
C2	0.2391 (20)	0.2550 (22)	0.6069 (6)
C3	0.2348 (24)	0.3107 (22)	0.6535 (6)
C4	0.2976 (28)	0.4573 (25)	0.6631 (7)
C5	0.3597 (22)	0.5565 (20)	0.6264 (7)
C6	0.3625 (29)	0.4994 (27)	0.5803 (7)
O4	0.3103 (15)	0.5023 (13)	0.7098 (5)
C1'	0.1964 (21)	0.6131 (20)	0.7266 (5)
C2'	0.2437 (23)	0.7103 (23)	0.7650 (6)
C3'	0.1239 (23)	0.8167 (20)	0.7833 (6)
C4'	-0.0262 (23)	0.8267 (20)	0.7656 (7)
C5'	-0.0701 (22)	0.7345 (24)	0.7289 (7)
C6'	0.0387 (28)	0.6229 (24)	0.7105 (6)
O4'	-0.1323 (16)	0.9376 (18)	0.7871 (5)
C7	0.3054 (27)	0.2871 (28)	0.5196 (7)
C8	0.2028 (23)	0.4035 (21)	0.4859 (6)
N8	0.0335 (20)	0.4221 (24)	0.5030 (6)
C9	0.2002 (24)	0.3401 (24)	0.4383 (7)
O10	0.0743 (20)	0.3335 (24)	0.4152 (6)
O9	0.3394 (17)	0.2961 (18)	0.4212 (4)
C10	0.3370 (40)	0.2414 (34)	0.3725 (8)

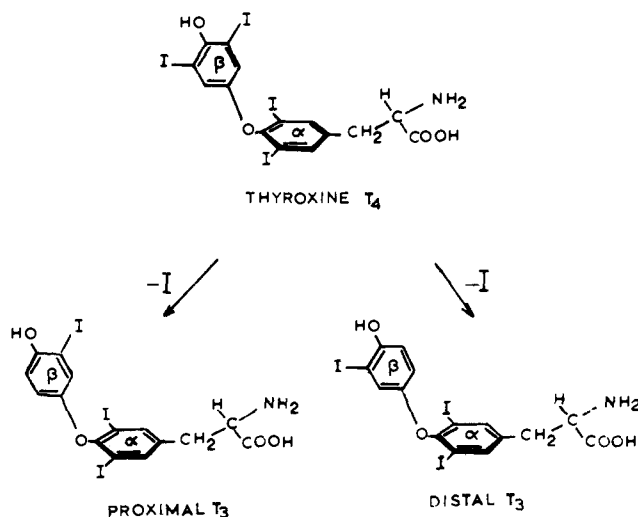
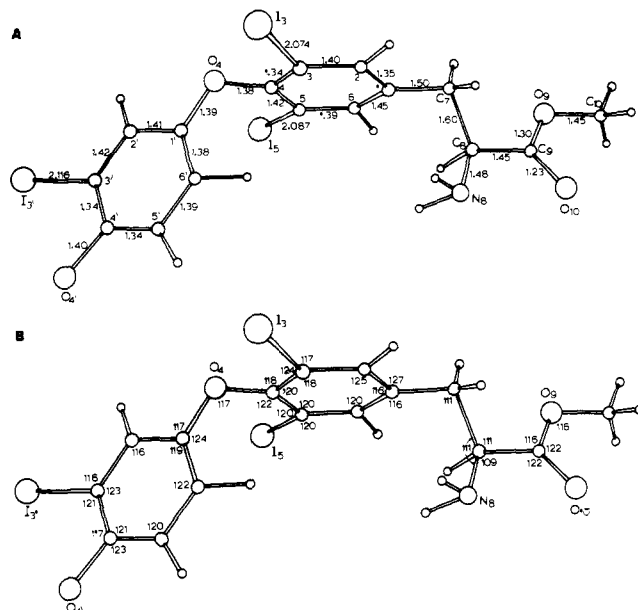
proximation to the least-squares normal equations. The structure refined readily to an *R* (defined as $\sum||F_o| - |F_c||/\sum|F_o|$) of 6.0%. The weighting scheme used in the final cycles of refinement was $w^{-1} = [1 + (|F_o| - 30/90)^2]$ which made the average weighted Δw^2 invariant with changing $|F_o|$.

Positional parameters for all nonhydrogen atoms are listed in Table II. Anisotropic thermal parameters for nonhydrogen atoms and hydrogen atom positional parameters, lists of the calculated structure factors, and detailed packing diagrams are available (see paragraph at end of paper regarding supplementary material).

Results and Discussion

All important intramolecular bonds and angles for 3,5,3'-triiodo-L-thyronine methyl ester are shown in Figure 2. The average standard deviations in bonds and angles are 0.028 Å and 1.7°, respectively, for all noniodine bonds and angles. The deviations for iodine-containing bonds and angles are 0.018 Å and 1.3°, respectively. While there are no bonds or angles which deviate by more than 2σ from their expected values, the bonds in the amino acid portion are alternately shorter and longer than the values observed in similar structures. The phenyl rings are planar and the maximum deviation of carbon atoms from the least-squares planes through the phenyl rings is 0.02 Å.

Thyroid Hormone Conformation. There are a number

**Figure 1.** Deiodination of thyroxine to either distal or proximal triiodothyronine.**Figure 2.** Bond distances (A) and bond angles (B) in 3,5,3'-triiodo-L-thyronine methyl ester. Hydrogen parameters were located from Fourier maps.

of conformational aspects of thyroid hormone structure which could play important roles in their transport, binding, and functional activity. These aspects include (1) amino acid conformation, (2) distal or proximal 3'-substituent, (3) diphenyl ether conformation, and (4) cisoid or transoid conformation. More detailed discussions of the first two points have been presented elsewhere.^{11b,17}

The diphenyl ether conformation can be defined by the torsional angles about the ether linkage, ϕ [C(5)-C(4)-O(41)-C(1')] and ϕ' [C(4)-O(41)-C(1')-C(6')]. These signed magnitudes indicate the direction of the angular deviation of the two phenyl rings from mutual perpendicularity. This is equivalent to the "H-inside" or ϕ and ψ both zero conformation in the notation used by Bergman, *et al.*¹⁸ In the ideal case ϕ and ϕ' are 90 and 0°, respectively.

An analysis of the results shown in Table IIIA reveals that the parameter ϕ is also a sensitive descriptor of the overall conformation of thyroid molecules, describing two conformations, cisoid or transoid, depending on whether ϕ is negative or positive, respectively. A negative ϕ , as ob-

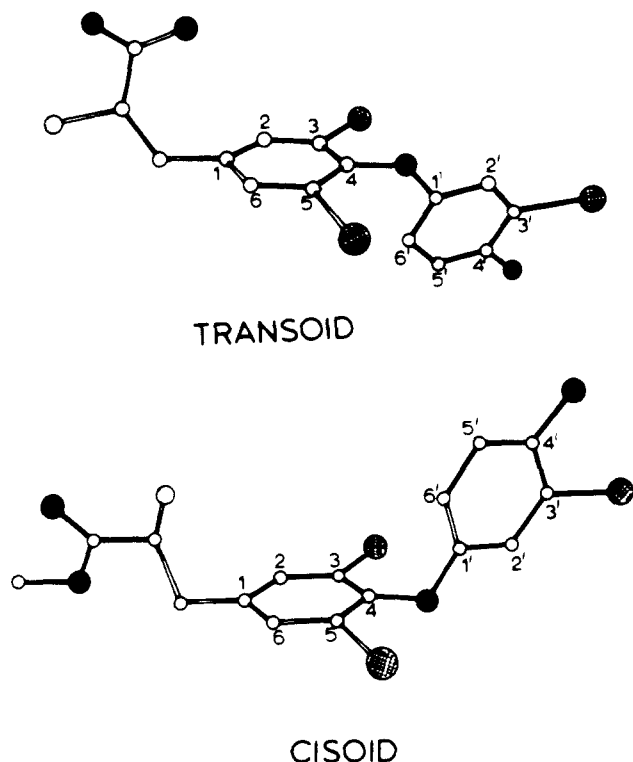


Figure 3. The cisoid or transoid conformations adopted by thyroid hormone structures.

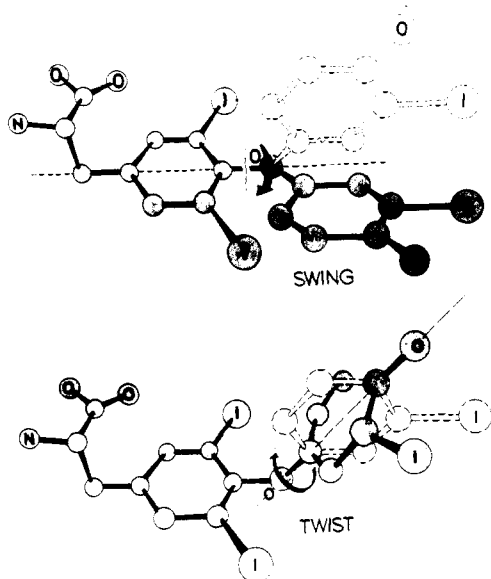


Figure 4. The diphenyl ether conformation showing the parameter ϕ describing the "swing" motion and ϕ' describing the "twist" motion of the two phenyl rings about the ether linkage.

served in this structure, delineates a conformation in which the outer phenyl ring and the alanine moiety lie on the same side (cisoid) of the inner ring plane, whereas a positive ϕ describes a conformation with these moieties on opposite sides of the inner ring plane (transoid) as illustrated in Figure 3.

Since there is no C^α carbon in the other diphenyl ether structures studied,²¹⁻²⁶ as listed in Table IIIB, the choice of cisoid or transoid for the overall description of these structures is arbitrary and the values of the torsional angles ϕ and ϕ' correspond to a transoid model observed for the conformation of many thyroid hormones and their analogs.

While the biological significance of the cisoid or tran-

Table III

A. Conformational Parameters for Thyroid Hormone Analogs (in deg)						
Structure	ϕ°	ϕ'°	χ^{1°	χ^{2°	Ψ^{1°	Ref
3,5,3'-Triiodo-L-thyronine methyl ester	-108	33	306	121	8	
3,5,3'-Triiodo-L-thyronine	116	-21	196	76	8	11b
3,5,3'-Triiodo-L-thyronine hydrochloride	90	-11	56	98	8	13
3,5-Diiodo-L-thyronine N-methylacetamide (1:1)	87	21	301	121	17	19
3,5,3',5'-Tetraiodo-L-thyronine hydrochloride	105	-34	67	98	321	20
B. Conformational Parameters for Diphenyl Ether Derivatives (in deg)						
Structure	ϕ	ϕ'	Ref			
2-(3'-Methyl-4'-nitrophenoxy)-1,3-diisopropylbenzene						
Molecule 1		80				5
Molecule 2		102				1 21
2-(2',4'-Dinitrophenoxy)-1,3,5-tri- <i>tert</i> -butylbenzene		94				2 22
2-(2'-Carbomethoxy-4'-nitrophenoxy)-1,3,5-trichlorobenzene						
Molecule 1		106				-10
Molecule 2		92				-5 23
2-(4'-Carbomethoxy-2'-nitrophenoxy)-1,3,5-trimethylbenzene		79				8 24
2,4-Dinitro-6-bromophenyl 1'-naphthyl ether		68				25 25
2,4-Dinitrophenyl 2',6'-diiodo-4'-methylphenyl ether		84				26 25
2,4-Dinitronaphthyl 2',6'-dimethylphenyl ether		60				22 26

$^a\phi = C(3)-C(4)-O(41)-C(1')$. $^b\phi' = C(4)-O(41)-C(1')-C(6')$. $^c\chi^1 = N(8)-C(8)-C(7)-C(1)$. $^d\chi^2 = C(8)-C(7)-C(1)-C(2)$. $^e\Psi^1 = O'-C'-C(8)-C(7)$.

Table IV. Hydrogen Bond and Intermolecular Contact Distances (Å) in 3,5,3'-Triiodo-L-thyronine Methyl Ester

O(41)···N(8)	2.69
I(5)···O(10)	2.99
I(3')···I(3)	4.19

soid conformation is not known, this property may well prove to have important biological implications. As illustrated in Figure 4, the torsional angle ϕ may be interpreted as a "swing" about the C(4)-O(41) axis. Similarly, the torsional angle ϕ' describes a "twist" about the O(41)-C(1') axis. Analysis of the parameters²⁷ in Table IIIA shows that the two motions are correlated; that is, in the cisoid conformation, if $\phi < -90^\circ$ then $\phi' < 0^\circ$ and if $\phi > -90^\circ$ then $\phi' > 0^\circ$. A reciprocal relationship is observed in the transoid conformation. However, perusal of these parameters in Table IIIB indicates that this correlation does not hold in all cases. The flexibility of the observed conformation of the structures in Table IIIB has the same range as that observed for thyronine structures.²⁷

Table IIIA also lists the conformational parameters which uniquely describe the amino acid backbone of thyroid hormones and their derivatives. The definitions of the

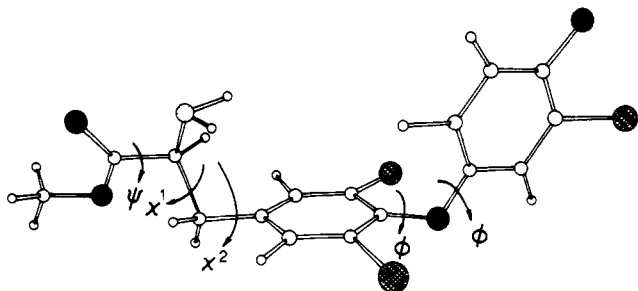


Figure 5. 3,5,3'-Triiodo-L-thyronine methyl ester conformation with rotational parameters defined.

torsional angles are in accord with the IUPAC-IUB nomenclature²⁸ and are illustrated in Figure 5. The parameters found in this, and other thyroid hormone analog structures, agree well with the preferred conformation adopted by amino acids as observed in a systematic analysis of 48 crystal structures containing aromatic amino acid residues with tyrosine or phenylalanine in their molecular make-up.¹⁷

Intermolecular Contacts. Each molecule of 3,5,3'-triiodo-L-thyronine methyl ester forms hydrogen bonds through the phenyl hydroxyl (Table IV). In addition, each molecule participates in a number of intermolecular contacts through the iodine atoms. The I...I contact of 4.19 Å, while shorter than the 4.30 Å predicted from the sum of van der Waals radii, is not unusually short compared to the average contact of 4.00 Å observed in other thyronine structures.²⁷ However, the I...O distance of 2.99 Å is extremely short compared with the expected value of 3.55 Å and is still significantly less than the average of 3.30 Å found in other thyronines.^{11b,19}

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Supplementary Material Available. A listing of structure factor amplitudes and the thermal parameters for the coordinates listed in Table II will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supple-

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