B· should not be cited as evidence for its nonplanarity.[56] Values of $a^{13}C_\alpha$ increase[57] along the series ·CH_3 (38.34 G),[58] $CH_3\dot{C}H_2$ (39.07 G),[58] cyclohexyl (41.3 G),[58] $B_2\dot{C}H$ (42.98 G), B· and $B_2\dot{C}CH_2MR_n$ (~46 G), and $B_3\dot{C}$ (51.10 G). It seems unlikely that a radical with such bulky ligands as $B_3\dot{C}$ could be anything but planar.

The low value of $a^{13}C_\alpha$ for the $(Me_3Si)_3\dot{C}$ radical (*ca.* 26 G)[59] has been interpreted in terms of a planar radical having significant delocalization of the unpaired electron (by analogy with $(C_6H_5)_3\dot{C}$ which has a similar $a^{13}C_\alpha$ value[60]). In this connection, it is worth noting that the s spin density on silicon is about 25% greater than that on the β carbons of $B_3\dot{C}$ but that the s spin density on the carbon and hydrogen of the methyl groups is only about half as great in $(Me_3Si)_3\dot{C}$ as in $B_3\dot{C}$.

$a^{13}C_\beta$ and Related Splittings. Hyperfine splittings by $^{13}C_\beta$ atoms in natural abundance have not, apparently, been previously observed. The $a^{13}C_\beta$ values found for $B_2\dot{C}H$ and $B_3\dot{C}$ are a bit smaller than those reported for (^{13}C enriched) simpler alkyl radicals, *viz.*, 13.57 G for ethyl,[58] 13.17 G for isopropyl,[3b] and 12.35 G for B.[3b] They are of similar magnitude to the $a^{13}C_\gamma$ values (as has been found in certain other radicals, *e.g.*, nitroxides[61] and semidiones[62]).

The $B_2\dot{C}Cl$ radical shows no unexpected epr spectroscopic features. That is, $a^{35}Cl$ is 2.6 G which is close to the values normally found for α-chloroalkyl radicals,[14,25,40,63] and the *g* factor is significantly larger than the free spin value as a result of the relatively large spin–orbit coupling constant for chlorine. It was not possible to resolve the lines due to ^{37}Cl from those due to ^{35}Cl.

Acknowledgment. We are grateful to Professor Kochi for a number of helpful comments and for letting us have a great deal of his unpublished data. We are also grateful to Professor Symons for his comments on, and interest in, this work.

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Crystal Structure and Molecular Conformation of the Thyroid Hormone Distal 3,5,3′-Triiodo-L-thyronine[1]

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Abstract: The crystal structure of the thyroid hormone 3,5,3′-triiodo-L-thyronine (T_3) has been determined and reveals the 3′-iodine in the distal position, *i.e.*, away from the alanine bearing ring. While this result had been anticipated from stereochemical and biological activity studies, previous crystallographic observations of structures in which the 3′-iodine was proximal had cast some doubt on the crystallographic stability of the 3′-distal conformation. The crystallographic observation of the two forms suggests that the relative energies of the two conformers are similar and that the barrier to internal rotation is not large. Recent molecular energy calculations on the barrier to rotation about the diphenyl ether linkage suggest that this is the case. The structure T_3 crystallizes in the monoclinic space group $P2_1$ with a = 13.891 (9), b = 5.999 (1), and c = 12.264 (3) Å and β = 116.81 (4)°. The final R index is 0.041. The planes of the two phenyl rings are skewed with respect to the ether C–O–C plane making angles of 115 and 21° with the inner and outer rings, respectively. The value of χ^1, the torsional angle about the C^α–C^β bond, which describes the amino acid backbone conformation, is 195°.

The question of the conformational preference of the thyroid hormone, triiodothyronine (T_3, Figure 1), has become increasingly important with the accumulation of recent evidence which suggests that this hormone 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 triiodothyronine in peripheral tissues.[2-4] The deiodination of T_4 yields two distinct conformers as illustrated in Figure 2, that is, a distal conformer in which the 3′-iodine is turned away from the inner ring and a proximal conformer with the 3′-iodine toward the inner ring. Because the 3′ and 5′ positions of the outer ring of T_3 are chemically equivalent but not conformationally equivalent, as shown by Jorgensen and

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his coworkers,[5,6] it had been uncertain whether it was the distal or proximal conformation which was preferred. The results of stereochemical and biological activity studies from several thyroxine analogs[7] are unambiguous in their indication that hormonal activity is greater for the distal orientation of the 3′-iodine. These findings were further substantiated in more recent studies of conformationally fixed thyroxine analogs to thyroxine binding globulin.[8]

While the molecular orbital energy calculations by Kollman,[9] using a modified CNDO/2 procedure, show the energy barrier to rotation about the diphenyl ether linkage to be small (≃11 kcal/mol), Kier and Hoyland[10] suggest a considerable barrier to internal rotation (50 kcal/mol). Neither study shows any significant preference for the distal or proximal conformer. These results are in disagreement with the molecular orbital calculations made by Camerman and Camerman[11] who find the total energy for the proximal T_3 to be lower than that computed for the distal T_3 (energy difference of 132 kcal).

Crystallographic observations of both the distal and proximal conformations of the thyroid hormone triiodo-L-thyronine and its analogs shed additional light on the question of the conformational preference of T_3. The solid-state observations of three structures [3,5,3′-triiodo-L-thyronine,[12] 3,5,3′-triiodothyroacetic acid–*N*-diethanolamine (1:1) complex,[13] and 3,5,3′-triiodo-L-thyronine methyl ester[14]] in which the 3′-iodine is distal and two structures (3,5,3′-triiodo-L-thyronine hydrate hydrochloride[11] and 3,5,3′-triiodothyropropionic ethyl ester[15]) in which the 3′-iodine is proximal suggest that these two conformers are indeed stable and readily accessible in solution. We report here the first complete structural details of a distally oriented 3′-iodine in the crystal and molecular structure of the pure active hormone 3,5,3′-triiodo-L-thyronine. 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, purchased from Sigma Chemicals, were grown at room temperature from an ethanol solution containing salicylic acid. A clear, well-formed crystal (0.4 × 0.16 × 0.04 mm³) was selected for intensity data collection. All X-ray measurements were made on a General Electric 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 ($0k0$, $k = 2n + 1$) indicated the monoclinic space group $P2_1$. The unit cell dimensions were obtained from a least-squares calculation based on 2θ measurements of 34 *hkl* reflections having $2\theta > 60°$ using Cu Kα radiation. The data were collected using Mo Kα radiation. The crystal data are presented in Table I.

The intensities of 1725 (1558 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 intensity of the $0\bar{4}0$ reflection ($\chi = 90°$, $2\theta = 27.36°$) varied up to a maximum of ±8% from its mean value within the ϕ range in which data were collected. Intensities were corrected for Lorentz and polarization factors, and an absorption correction based on the intensity variation of ϕ at $\chi = 90°$ was applied.

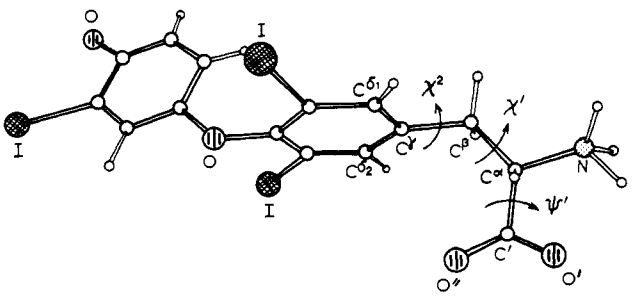

Figure 1. 3,5,3′-Triiodo-L-thyronine (T_3) with conformational parameters defined.

Table I. Crystal Data for 3,5,3′-Triiodo-L-thyronine

Formula	$C_{15}H_{12}O_4NI_3$
Mol wt	648.0
Space group	$P2_1$
a	13.891 (4) Å
b	5.999 (1) Å
c	12.264 (3) Å
β	116.81 (4)°
V	912.1 Å³
d_{calcd}	2.35 g cm^{-3}
Z	2
Crystal size	0.40 × 0.16 × 0.04 mm³
R	4.1%
R_w	4.4%

Structure Determination and Refinement. The structure was solved by the application of heavy atom techniques. The iodine atoms were located from Patterson functions and the complete structure from three-dimensional Fourier synthesis. After four cycles of isotropic diagnonal least-squares refinement, the *R* index ($R = \Sigma||F_o| - |F_c||/\Sigma F_o$) was 0.10. Further cycles of anisotropic refinement of the nonhydrogen atoms by block-diagonal least-squares refinement reduced the *R* index to 0.049.

Although a three-dimensional Fourier difference map, calculated without the hydrogen atom contributions to the structure factors, produced well-defined electron densities for 8 of the 12 hydrogen atoms, their geometry was not acceptable so the hydrogen atoms were placed at their theoretically predicted positions. The hydrogen positional and thermal parameters were held constant throughout further refinement cycles with the thermal parameters fixed at 3.0 Å².

All scattering factors were taken from the International Tables for X-Ray Crystallography (1962), and the real and imaginary parts of the anomalous dispersion correction applied to the iodine atoms. The difference in the resulting *R* values for the plus and minus anomalous dispersion correction (0.051 and 0.059) confirmed that the molecule was in the L configuration.

The weighting scheme throughout the final cycles of refinement was $w^{-1} = \{1 + [|F_o| - 65/30]^2\}^{1/2}$ with the weighting constants evaluated to make $w\Delta^2$ invariant with changing $|F_o|$. Refinement terminated with $\Sigma w(|F_o| - |F_c|)^2/m - n$, the "goodness of fit," at 1.8. The final *R* value was 4.1% (Table I).

The final fractional coordinates and anisotropic thermal parameters for the triiodothyronine structure are given in Table II. The fractional coordinates for the theoretical hydrogen positions are listed in Table III. (Observed and calculated structure factors can be obtained from the author upon request.)

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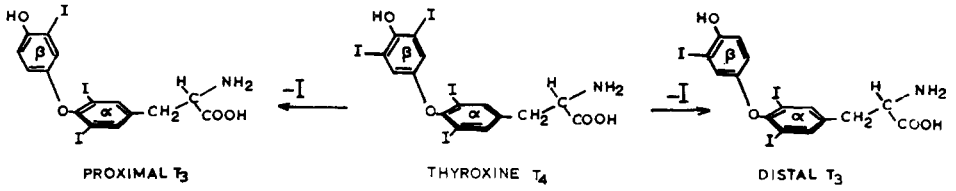

Figure 2. Deiodoination of thyroxine to either a distal or proximal triiodothyronine.

Table II. Positional and Thermal Parameters of the Nonhydrogen Atoms of Triiodo-L-thyronine[a]

	x/a	y/b	z/c	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
I(3)	1.1571 (0)	0.2500 (2)	1.0339 (0)	0.0326 (5)	0.0420 (6)	0.0331 (5)	0.0051 (6)	0.0103 (4)	−0.0026 (6)
I(5)	0.9893 (0)	0.8818 (2)	0.5968 (1)	0.0372 (5)	0.0360 (5)	0.0457 (6)	0.0007 (6)	0.0231 (4)	0.0076 (6)
I(3′)	1.4853 (1)	1.1173 (2)	0.8815 (1)	0.0449 (6)	0.0475 (7)	0.0538 (7)	−0.0224 (6)	0.0256 (5)	−0.0141 (7)
C(1)	0.8587 (12)	0.3183 (30)	0.7024 (14)	0.0284 (80)	0.0417 (99)	0.0329 (87)	−0.0061 (73)	0.0155 (70)	−0.0019 (76)
C(2)	0.9410 (12)	0.2580 (39)	0.8164 (13)	0.0375 (87)	0.0408 (99)	0.0339 (85)	−0.0091 (99)	0.0214 (72)	0.0010 (99)
C(3)	1.0364 (11)	0.3718 (35)	0.8680 (13)	0.0244 (74)	0.0451 (99)	0.0245 (77)	−0.0027 (90)	0.0130 (63)	−0.0009 (91)
C(4)	1.0519 (10)	0.5580 (29)	0.8074 (14)	0.0171 (77)	0.0344 (98)	0.0382 (87)	0.0013 (70)	0.0200 (71)	−0.0005 (74)
C(5)	0.9729 (14)	0.6184 (33)	0.6939 (15)	0.0490 (98)	0.0336 (98)	0.0358 (91)	0.0052 (95)	0.0228 (80)	0.0148 (93)
C(6)	0.8774 (11)	0.5031 (31)	0.6444 (14)	0.0169 (71)	0.0302 (91)	0.0287 (82)	0.0013 (72)	0.0063 (64)	0.0023 (76)
C(7)	0.7555 (11)	0.1786 (29)	0.6427 (15)	0.0141 (69)	0.0404 (99)	0.0360 (91)	−0.0027 (72)	−0.0001 (65)	−0.0018 (80)
C(8)	0.6774 (11)	0.2150 (28)	0.6976 (14)	0.0202 (71)	0.0216 (92)	0.0380 (87)	−0.0111 (69)	0.0119 (65)	−0.0023 (76)
N(8)	0.5973 (9)	0.0279 (26)	0.6574 (11)	0.0184 (64)	0.0362 (85)	0.0336 (73)	0.0084 (62)	0.0122 (57)	0.0059 (66)
C(9)	0.6211 (11)	0.4372 (27)	0.6659 (15)	0.0186 (72)	0.0266 (99)	0.0487 (98)	−0.0031 (66)	0.0208 (70)	−0.0061 (77)
O(10)	0.6770 (8)	0.6044 (20)	0.7094 (10)	0.0294 (57)	0.0179 (61)	0.0561 (74)	−0.0025 (56)	0.0097 (54)	0.0018 (62)
O(9)	0.5208 (8)	0.4388 (19)	0.6019 (10)	0.0245 (55)	0.0235 (70)	0.0467 (67)	0.0083 (47)	0.0157 (51)	−0.0006 (53)
O(4)	1.1459 (7)	0.6766 (21)	0.8667 (10)	0.0224 (53)	0.0491 (92)	0.0453 (67)	−0.0206 (54)	0.0244 (50)	−0.0097 (58)
C(1′)	1.2224 (11)	0.6868 (35)	0.8183 (16)	0.0085 (68)	0.0707 (99)	0.0484 (99)	−0.0001 (78)	0.0136 (68)	0.0158 (96)
C(2′)	1.2920 (11)	0.8593 (32)	0.8562 (12)	0.0188 (69)	0.0472 (99)	0.0202 (71)	−0.0066 (78)	0.0136 (59)	−0.0118 (79)
C(3′)	1.3654 (11)	0.8839 (33)	0.8113 (13)	0.0247 (76)	0.0330 (93)	0.0331 (82)	−0.0206 (88)	0.0104 (66)	−0.0000 (90)
C(4′)	1.3654 (10)	0.7292 (33)	0.7224 (13)	0.0208 (70)	0.0366 (99)	0.0260 (75)	0.0047 (79)	0.0092 (60)	−0.0016 (83)
C(5′)	1.2947 (14)	0.5582 (32)	0.6889 (17)	0.0354 (94)	0.0374 (99)	0.0497 (99)	0.0020 (85)	0.0172 (86)	−0.0125 (92)
C(6′)	1.2202 (12)	0.5266 (34)	0.7350 (14)	0.0235 (79)	0.0411 (99)	0.0285 (81)	−0.0155 (79)	0.0109 (67)	−0.0122 (78)
O(4′)	1.4394 (8)	0.7623 (28)	0.6790 (10)	0.0359 (61)	0.0453 (75)	0.0600 (77)	−0.0126 (72)	0.0307 (58)	−0.0098 (84)

[a] Temperature factors are of the form $\exp[-2\pi^2(U_{11}h^2a^{*2} + 2U_{12}hka^*b^* + \cdots)]$.

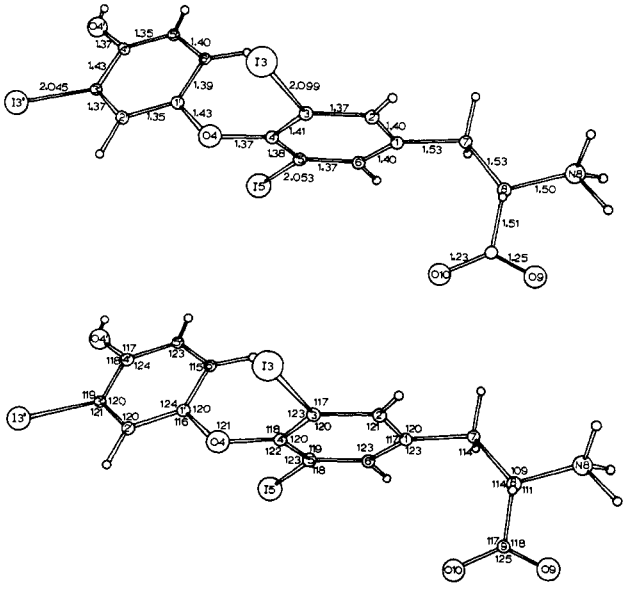

Figure 3. Bond distances and bond angles for 3,5,3′-triiodo-L-thyronine.

Molecular Geometry

The bond lengths and angles calculated from the coordinates in Table II are given in Figure 3. The estimated standard deviations for the C–C bond lengths of the triiodo-L-thyronine molecule range from 0.015 to 0.026 Å with an average value of 0.02 Å, while e.s.d.'s for the corresponding bond angles range between 0.6 and 1.5° with an average value of 1.1°. Most observed bond lengths and angles are within one standard deviation of their expected values, with only three exceptions in which aromatic C–C bond lengths differ by standard deviations from the expected value of 1.397 Å.

The carbon–iodine distances of 2.099, 2.053 and 2.045 Å in this structure agree very well with the average value of 2.05 Å observed in other aromatic iodinated structures compiled in Tables of Interatomic Distances (1965).[16] The average C–I bond length found in other thyronine structures is 2.095 Å.

Table III. Atomic Positional Parameters for the Hydrogen Atoms of Triiodo-L-thyronine

	x/a	y/b	z/c
Th(2)	0.929	0.119	0.865
Th(6)	0.814	0.556	0.557
TH(7A)	0.778	0.005	0.653
TH(7B)	0.714	0.221	0.547
TH(B)	0.723	0.206	0.796
THN(A)	0.639	−0.128	0.689
THN(B)	0.553	0.026	0.559
THN(C)	0.542	0.054	0.696
TH(2′)	1.290	0.977	0.922
TH(5′)	1.296	0.440	0.623
TH(6′)	1.165	0.388	0.708
TO(4′)	1.415	0.794	0.610

(16) "Table of Interatomic Distances and Configurations in Molecules and Ions," *Chem. Soc., Spec. Publ., Suppl.*, No. 18 (1965).

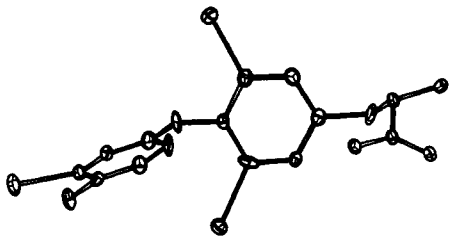

Figure 4. 3,5,3′-Triiodo-L-thyronine showing 26% probability thermal ellipsoid plots.

The equivalent carboxyl–oxygen distances of 1.23 and 1.25 Å in the amino acid portion indicate that the molecule is in its zwitterion form. The bond lengths and angles for the alanine portion also agree well with values observed in other structures. There is no significant difference in the lengths of the ether linkages.

The relative thermal motion of triiodothyronine is shown in Figure 4. Minor deviations of the iodine and oxygen atoms from the best least-squares plane through the phenyl rings are listed in Table IV.

Table IV. Deviations from the Least-Squares Plane through the Two Phenyl Rings of 3,5,3′-Triiodo-L-thyronine

Planes through first six atoms			
Atom	Distance, Å	Atom	Distance, Å
C(1)	0.0019	C(1′)	−0.0082
C(2)	−0.0020	C(2′)	−0.0030
C(3)	0.0084	C(3′)	0.0125
C(4)	−0.0148	C(4′)	−0.0107
C(5)	0.0148	C(5′)	0.0000
C(6)	−0.0084	C(6′)	0.0095
C(7)	0.0845	I(3′)	0.2664
I(3)	0.1367	O(4′)	−0.0113
I(5)	0.0284	O(4)	−0.0665
O(4)	−0.0999		

Tyrosine Conformation

Table V lists the conformational parameters used to describe the backbone conformation of the tyrosine portion of six thyronine derivatives. The definitions of the torsional angles are in accord with the IUPAC–IUB nomenclature[17] and are illustrated in Figure 1. From a systematic analysis[18] of 48 crystal structures of aromatic amino acids or amino acid residues with tyrosine or phenylalanine in their make-up, it has been shown that there are three basic energy minimum conformations which these molecules adopt. These groups can be further subdivided into subsets defined by the interactions of the three rotational parameters χ^1, χ^2, and ψ^1.

Table V. Conformational Parameters for Thyroid Compounds

Compound	χ^1, deg[a]	χ^2, deg[b]	ψ^1, deg[c]	Ref
3,5,3′-Triiodo-L-thyronine	196	76	353	This work
3,5,3′-Triiodo-L-thyronine methyl ester	306	121	8	14
3,5,3′-Triiodo-L-thyronine hydrochloride	56	98	8	11
3,5-Diiodo-L-thyronine–*N*-methylacetamide (1:1)	301	121	17	21
3,5,3′,5′-Tetraiodo-L-thyronine hydrochloride	67	98	321	*d*
L-Thyronine ethyl ester	66	90	353	*e*

[a] χ^1 is the torsional angle N–C^α–C^β–C^γ. [b] χ^2 is the torsional angle C^α–C^β–C^γ–C^δ. [c] ψ^1 is the torsional angle O′–C′–C^α–N (cis). [d] N. Camerman and A. Camerman, *Proc. Nat. Acad. Sci. U. S.*, **69**, 2130 (1972). [e] A. Camerman, private communication.

(17) IUPAC–IUB Commission on Biochemical Nomenclature: *J. Mol. Biol.*, **52**, 1 (1970).

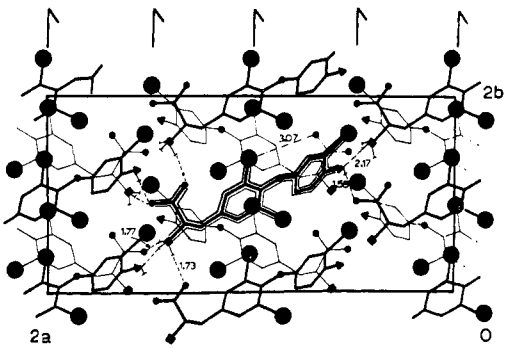

Figure 5. Packing diagram for 3,5,3′-triiodo-L-thyronine, with the hydrogen bonding scheme outlined. The large circles are iodine, the squares nitrogen, and the circles oxygen atoms. The dashed lines indicate hydrogen bonded distances from the hydrogen atom to the nitrogen or oxygen, respectively. The dark molecules are above the light ones. Also shown is the iodine–oxygen contact distance.

The parameter χ^1(N–C^α–C^β–C^γ) has three preferred values: ≃60, 180, and 300°. All three staggered conformations are observed for the thyroid hormones. The expected value, corresponding to the minimum energy conformation, for the parameter χ^2(C^α–C^β–C^γ–C^δ) is approximately 90°. While the χ^2 values in Table V range from 76 to 121° and have an average of 101°, these values are correlated in the same way with the parameter ψ^1 as previously observed in the earlier systematic analysis of aromatic amino acids.[18] The conformations of the tyrosine moiety of the thyronine derivatives in Table V are fully consistent with the observations and correlations cited in the survey.[18]

Crystal Packing and Hydrogen Bonding

Four distinct hydrogen bonds (Table VI and Figure 5) are the primary intermolecular contacts in the structure of 3,5,3′-triiodo-L-thyronine. Each molecule of T_3 is hydrogen bonded to eight others. Six of these molecular contacts bridge translationally related molecules which form layers perpendicular to the *c* direction. The remaining hydrogen bonds connect amino acids related by a screw axis "tying" the layers together.

Table VI. Hydrogen Bonds in Triiodo-L-thyronine

	X–H, Å	H···Y, Å	X···Y, Å	∠X–H···Y, deg
O(4′)···O(9)	1.05	1.58	2.63	180
N(8)–H(A)···O(10)	1.09	1.73	2.73	149
N(8)–H(B)···O(9)	1.09	1.80	2.89	146
N(8)–H(C)···O(4′)	1.07	2.18	2.81	116

There are two iodine contacts of interest. The intermolecular contact between two iodine atoms of 3.92 Å is significantly less than the normal van der Waals

(18) V. Cody, W. L. Duax, and H. Hauptman, *Int. J. Peptide Protein Res.*, **5**, 308 (1973).

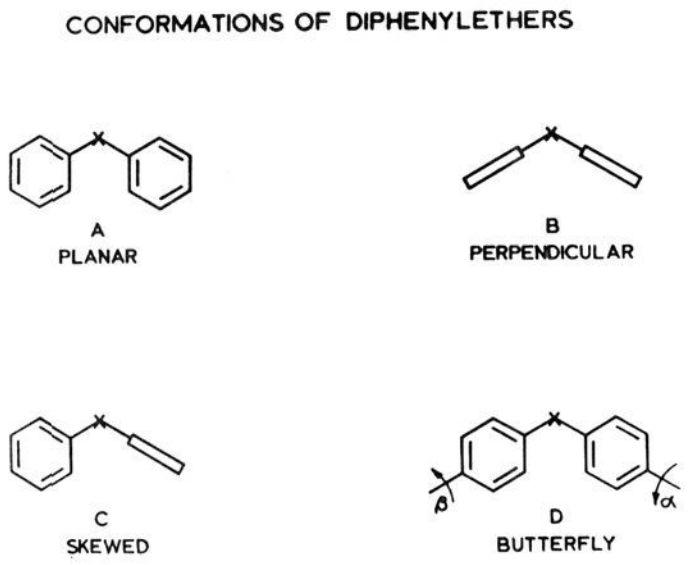

Figure 6. Four conformations considered for diphenyl ethers.

distance of 4.30 Å of Pauling[19] but nearer the 4.08Å distance of Bondi.[20] The other contact is an iodine–oxygen (I···O=C) distance of 3.07 Å which is significantly shorter than either Pauling's or Bondi's value of 3.55 or 3.48 Å, respectively. This type of close contact between an inner-ring iodine and a carboxylic oxygen has also been observed in the diiodothyronine[21] structure. The presence of these short iodine–iodine interactions appears to be a common feature of thyroxine analogs. The 3′-iodine is not involved in the short I···I interactions.

Diphenyl Ether Conformation

Four conformationally distinct models for diphenyl ethers have been proposed in discussions of the interpretation of dipole moments, dielectric relaxation, and nmr measurements in solution.[22–25] These conformers are illustrated in Figure 6. Of the four models proposed for unsubstituted diphenyl ethers, only two (C and D) have been observed in thyroid compounds. Diortho substitution appears to restrict conformational freedom. The rotational parameters used to describe model C (skewed) which has one ring coplanar with and the other perpendicular to the plane of the two carbon–oxygen bonds are $\phi = 90°$, $\phi' = 0°$ in the ideal case, whereas in model D (butterfly) both phenyl rings are free to rotate about the carbon–oxygen bonds by the angles ϕ and ϕ' out of coplanarity with the C–O–C plane.

In general, the diphenyl ether conformations have been reported in terms of the two dihedral angles which are the angles between the ether C–O–C plane and the least-squares planes of the two phenyl rings, respectively. However, more informative parameters are the torsional angles about the ether linkage. The two angles, ϕ [C(5)–C(4)–O(4)–C(1′)] and ϕ' [C(4)–O(4)–C(1′)–C(6′)] (Figure 3), will unambiguously describe the relative orientation of the two rings and, because they may be positive or negative, their values will give

(19) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1960.
(20) A. Bondi, *J. Phys. Chem.*, **68**, 441 (1964).
(21) V. Cody, W. L. Duax, and D. A. Norton, *Acta Crystallogr., Sect. B*, **28**, 2244 (1972).
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(25) R. J. W. LeFevre and J. D. Saxby, *J. Chem. Soc. B*, 1064 (1966).

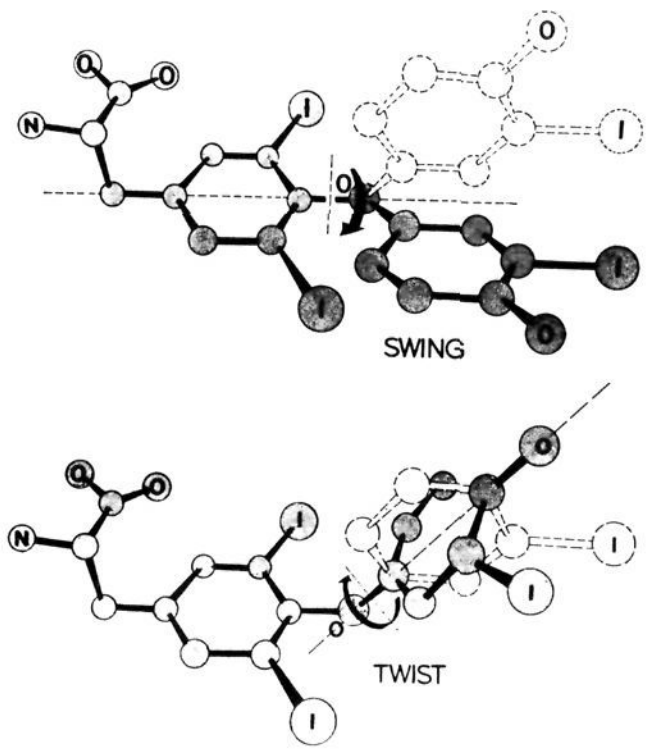

Figure 7. The diphenyl ether conformation showing the parameters ϕ describing the "swing" motion and ϕ' describing the "twist" motion of the two phenyl rings about the ether linkage.

the direction of the angular deviation of the two phenyl rings from mutual perpendicularity. As illustrated in Figure 7, the first torsional angle ϕ may be interpreted as a "swinging" about the axis C(4)–O(4). Similarly, the second torsional angle ϕ' describes a "twisting" about the axis O(4)–C(1′). Analysis of these parameters[26] shows that the two motions are correlated.

As noted in Table VII, the observed rotational parameters in T_3 show the greatest deviation from mutual perpendicularity (116°/−21°) of those active thyroid hormones or analogs studied by X-ray analysis. While these results suggest that the preferred conformation of substituted diphenyl ethers is skewed, the variation in observed values shows that a significant degree of conformational flexibility is allowable.

Table VII. Torsional Angles between Diphenyl Ether Linkages

Structure	ϕ [C(5)–C(4)–O(4)–C(1′)], deg	ϕ' [C(4)–O(4)–C(1′)–C(6′)], deg	Ref
3,5,3′-Triiodo-L-thyronine	116	−21	This work
3,5,3′-Triiodo-L-thyronine methyl ester	−108	33	14
3,5,3′-Triiodothyroacetic acid–*N*-diethanolamine (1:1)	88	5	13
3,5,3′-Triiodo-L-thyronine hydrochloride	90	−11	11
3,5-Diiodo-L-thyronine–*N*-methylacetamide (1:1)	87	21	21
3,5,3′,5′-Tetraiodo-L-thyronine hydrochloride	105	−34	*a*

[a] See footnote *e*, Table V.

Discussion

The repeated crystallographic observation of both the distal and proximal conformers of the thyroid hormone T_3 indicates that both forms are readily available in solution and that by judicious changes in crystallizing

(26) V. Cody, Pittsburgh Diffraction Conference, Pittsburgh, Pa., Nov 1973.

conditions either conformer can be crystallized. The ease with which these conformers can be crystallized suggests that their energy difference and/or barrier to internal rotation are relatively small, and this has been corroborated by the most recent MO energy calculations.[9] That conformation is relevant to function, for thyroid hormones, is accepted, but just *how* is not clearly defined. Structure–functional analysis of thyroid hormones must take into account three important conformational facts: (1) both the distal and proximal orientations of a 3′ substituent are possible in solution, (2) there is a definite pattern in the flexibility of the two phenyl rings observed in the diphenyl ether conformation, and (3) there is a predictable degree of variability in the amino acid conformation.

The conformation observed in this structure agrees with the *in vivo* results which show a need for a distally oriented substituent for activity. The *in vivo* requirements for the amino acid conformation are still uncertain.

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Cyclic Peptides. IX. Conformations of a Synthetic Ion-Binding Cyclic Peptide, *cyclo*-(Pro-Gly)$_3$, from Circular Dichroism and ^{1}H and ^{13}C Nuclear Magnetic Resonance[1]

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Abstract: The solution conformers of a synthetic ion-binding cyclic hexapeptide, *cyclo*-(L-prolyl-glycyl)$_3$ [*cyclo*-Pro-Gly)$_3$], are derived from the complementary information of circular dichroism (CD) spectra, ^{1}H and ^{13}C nuclear magnetic resonance (nmr) spectra, theoretical CD spectra, and computed intramolecular potential energies. In polar solvents, such as water and dimethyl sulfoxide, *cyclo*-(Pro-Gly)$_3$ adopts an asymmetric conformation which contains one cis Gly-Pro peptide bond. In the nonpolar solvents, dioxane and chloroform, the *cyclo*-(Pro-Gly)$_3$ conformer is C_3 symmetric, has all peptide bonds trans, and is stabilized by three 1 ← 3 hydrogen bonds (γ turns). *cyclo*-(Pro-Gly)$_3$–cation complexes form a second class of all-trans C_3-symmetric conformers which do not contain intramolecular hydrogen bonds. Among the alkali metal cations, *cyclo*-(Pro-Gly)$_3$ shows selectivity for Li^+ and Na^+ over K^+ and larger cations. Among the divalent alkaline earths of corresponding ionic radii, *cyclo*-(Pro-Gly)$_3$ selectively binds Mg^{2+} and Ca^{2+} in preference to Ba^{2+}. The measured *cyclo*-(Pro-Gly)$_3$–cation binding constants are comparable to those observed for related, naturally occurring cyclic peptides. For example, the binding constant of *cyclo*-(Pro-Gly)$_3$ for Ca^{2+} in acetonitrile is 1.1×10^5 M^{-1}. Magnesium forms three distinct complexes with *cyclo*-(Pro-Gly)$_3$ with stoichiometries of Mg^{2+}:*cyclo*-(Pro-Gly)$_3$ of 1:2, 1:1, and 2:1.

The effects of amino acid sequence, solvent, and temperature on the stability of peptide structures can be evaluated through the study of synthetic cyclic peptides whose entire conformational space can be explored. A family of cyclic peptides, *cyclo*-(L-prolyl-glycyl)$_n$ (n = 1, 2, 3, . . .), provides excellent models for such studies of conformational determinants. Since members of the *cyclo*-(Pro-Gly)$_n$ family bind cations, they are also valuable in assessing relative binding strengths and selectivities for a series of biologically relevant cations.

Previous ^{1}H and ^{13}C nuclear magnetic resonance (nmr) studies have identified three conformational classes for *cyclo*-(L-prolyl-glycyl)$_3$ [*cyclo*-(Pro-Gly)$_3$].[2,3] Two of these conformers are C_3 symmetric, as evidenced by the magnetic equivalence of the three Pro-Gly units. However, the third conformer is asymmetric and gives separate resonances for each Pro-Gly unit. The deduced symmetries, proton–proton coupling constants, and Corey–Pauling–Koltun (CPK) molecular models were used to make preliminary conformational assignments.[2,3] These earlier studies indicated likely conformational states, but the data were not sufficient to define each of the three conformers in detail.

In a subsequent theoretical study,[4] the intramolecular potential energy (the sum of van der Waals and dipolar interactions) was computed for all possible *cyclo*-(Pro-Gly)$_3$ conformers. Utilizing standard coordinates for the peptide units, this investigation considered only dihedral angle variations. In addition, only trans Pro-Gly peptide bonds were considered, since the trans isomer for secondary peptide groups is at least 2 kcal/mol more stable than the cis isomer.[5] In contrast, both

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