Molecular Structure of Thyroxine Analogues. Crystal Structure of 3,5,3′-Triiodothyroacetic and 3,5,3′,5′-Tetraiodothyroacetic Acid *N*-Diethanolamine (1:1) Complexes[1]

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Crystallographic data demonstrate that conformations of thyroid hormones and their derivatives in which the phenyl rings are either skewed (ϕ,ϕ'; ±90,0°) or twist-skewed (ϕ,ϕ'; ±108, ∓28°) are energetically favored. Acetic acid metabolites are consistently observed in the skewed conformation whereas their parent hormones are observed in the twist-skewed conformation. These preferences are manifestations of long-range conformational transmission and together with plasma protein binding data may indicate a site-specific preference for the skewed vs. twist-skewed conformation. These findings result in part from the crystal structure determinations of the *N*-diethanolamine (1:1) complexes of the active thyroxine metabolites 3,5,3′-triiodothyroacetic acid (T_3AA) and 3,5,3′,5′-tetraiodothyroacetic acid (T_4AA) which are reported here. The conformation of the 3′-iodine in the hypocholestermic agent T_3AA is distal, the biologically preferred conformation, and the overall conformation of T_3AA is transoid, while that of T_4AA is cisoid.

On oral administration the acetic acid analogues of thyroxine (T_4) and triiodothyronine (T_3) are less than one-tenth as active as their parent compounds.[2] In man, the plasma half-times are considerably shorter than the half-times of the parent compounds. This is in contrast to the observation that, despite its low hormonal potency, triiodothyroacetic acid (T_3AA) is bound at least as strongly to rat hepatic nuclei as is T_3 and binds 16 times more strongly to plasma proteins.[3,4] One explanation for these discrepancies is that a more rapid metabolism of T_3AA results in a shorter exposure to the hepatic nuclei to this analogue. These binding studies suggest that the presence of the α-amino group of T_3 and T_4 may inhibit binding to a number of proteins.

Recent developments of radioimmunoassay techniques[5] for T_3AA and 3,5,3′,5′-tetraiodothyroacetic acid (T_4AA) have permitted studies concerning the conversion of T_4AA to T_3AA. These data suggest that, like T_4, T_4AA is deiodinated to T_3AA which exerts the major metabolic effect of the acetic acid analogues.

The crystal structures of the acetic acid metabolites of T_3 and T_4 (Figure 1) were undertaken in order to determine whether they differ significantly from the parent hormones and whether any conformational patterns exist which can be correlated with the observed differences in binding and metabolism.

Experimental Section

Crystals of both 3,5,3′-triiodothyroacetic acid and 3,5,3′,5′-tetraiodothyroacetic acid were grown at room temperature from methanol solutions containing *N*-diethanolamine. Samples of T_3AA were purchased from Sigma Chemical Co. as the *N*-diethanolamine salt. Smith Kline & French provided the T_4AA. The crystal data for both complexes are listed in Table I.

Both structures were solved by standard heavy atom techniques and refined anisotropically by full-matrix least-squares techniques to a final *R* index of 0.098 and 0.10 for T_4AA and T_3AA, respectively. Positional and anisotropic thermal parameters for all nonhydrogen atoms of both structures, diagrams illustrating observed bond lengths and angles, lists of the calculated structure factors, and detailed packing diagrams are available (see paragraph at end of paper regarding supplementary material).

Results

In T_3AA the 3′-iodine is in the distal conformation (away from the inner ring), previously observed in the crystal structures of triiodothyronine[6] and its methyl ester.[7] The diphenyl ether conformation is defined by the torsion[8] angles ϕ [C(5)–C(4)–O(4)–C(1′)][15] and ϕ' [C(4)–O(4)–C(1′)–C(6′)], and the conformation of the acetic acid moiety relative to the phenyl ring is defined by the torsion angle

Table I. Crystal Data for 3,5,3′-Triiodo- and 3,5,3′,5′-Tetraiodothyroacetic Acid *N*-Diethanolamine (1:1) Complexes

	Triiodothyroacetic acid	Tetraiodothyroacetic acid
Mol formula	$C_{14}H_9O_4I_3 \cdot C_4H_{11}O_2N$	$C_{14}H_8O_4I_4 \cdot C_4H_{11}O_2N$
Mol wt	727.08	852.97
Crystal system	Monoclinic	Monoclinic
Space group	*C*2/*c*	*C*2/*c*
Z	8	8
Cell dimensions		
a, Å	29.21 (2)	24.284 (3)
b, Å	8.048 (3)	8.869 (4)
c, Å	20.48 (2)	23.693 (4)
β, deg	107.13 (2)	114.22 (1)
Vol, Å^3	4595.2	4653.6
Density (calcd), g/cm^3	2.10	2.44
Crystal size, mm	0.4 × 0.3 × 0.2	0.4 × 0.6 × 0.2
λ, Å	0.7107	0.7107
μ, cm^{-1}	41.6	54.5
R, %	10.0 (2553 obsd data)	9.8 (4543 obsd data)

χ^2 [C(2)–C(1)–C(7)–C(8)]. The values of these parameters in T_3AA and T_4AA are listed in Table II and are compared with analogous parameters in T_3, T_4, and their derivatives.

In both T_3AA and T_4AA the phenyl rings are skewed (ϕ,ϕ'; ±90,0°). As illustrated in Figure 2, the general description of T_3AA is transoid (ϕ positive) with the outer phenyl ring and the acetic acid moiety on opposite sides of the inner ring plane, and in T_4AA it is cisoid (ϕ negative) with these moieties on the same side of the inner phenyl ring plane. From the plot of the two torsion angles ϕ and ϕ' in Figure 3, it can be observed that not only is the parameter ϕ a sensitive descriptor of overall geometry,[9] but the distribution of cisoid and transoid conformers among the 15 thyroactive crystal structures[10] is also nearly equal. Figure 3 also shows that, for each the cisoid and transoid conformers, ϕ and ϕ' are linearly related.

The acetic acid side chain (χ^2) in both structures deviates from perpendicularity to the inner ring plane, the energy minimum conformation predicted for aromatic amino acids.[11] However, the observed deviations from 90° are within the range of χ^2 values found in other thyroid hormone structures.[9,11] The carboxylic acid function is nearly coplanar with the side chain in T_4AA but is 33° out of the plane in T_3AA (Table II).

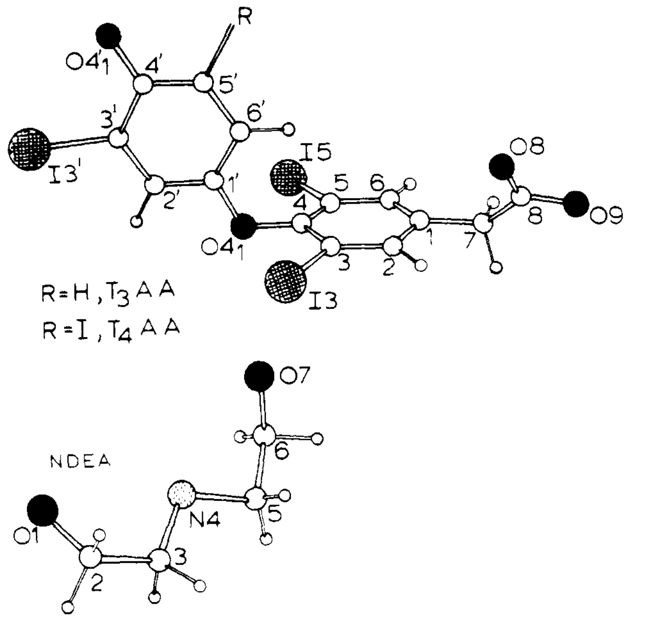

Figure 1. Thyroacetic acid *N*-diethanolamine (1:1) complex with numbering scheme used: when R = H, 3,5,3'-triiodothyroacetic acid; when R = I, 3,5,3',5'-tetraiodothyroacetic acid.

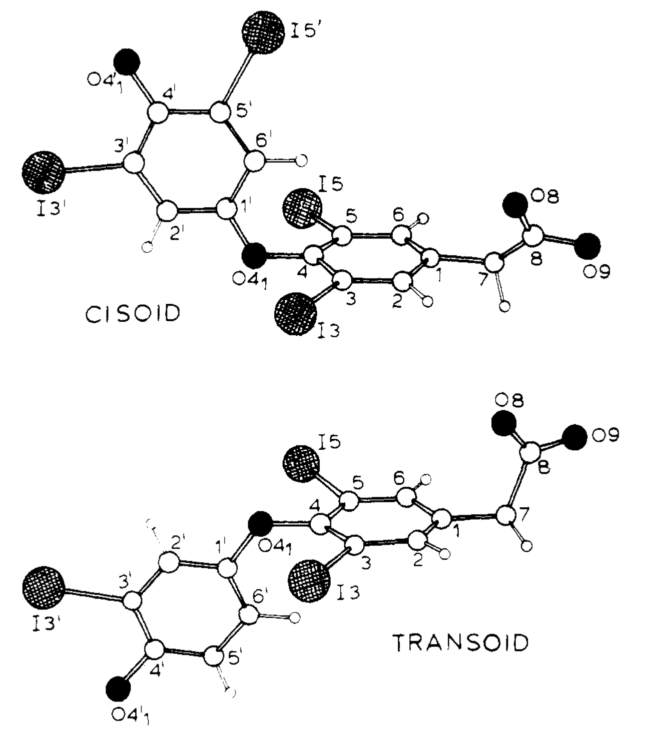

Figure 2. T_3AA and T_4AA illustrating the transoid and cisoid conformation, respectively.

Discussion

Comparison of T_3AA and T_4AA with each other and with their parent hormones reveals a specific influence of side-chain composition upon the diphenyl ether conformation and good conformational overlap between the thyroacetic acid metabolites and their parent hormones in which the α-amino acid function is fully extended. These conformational patterns have implications concerning the binding characteristics and activities of these compounds.

Substitution of the acetate for the amino acid side chain does not effect χ^2 but does appear to have long-range effects on the diphenyl ether conformation (ϕ and ϕ'). The two acetic acid orientations are comparable to the side-chain orientations observed in the active thyroid hormone

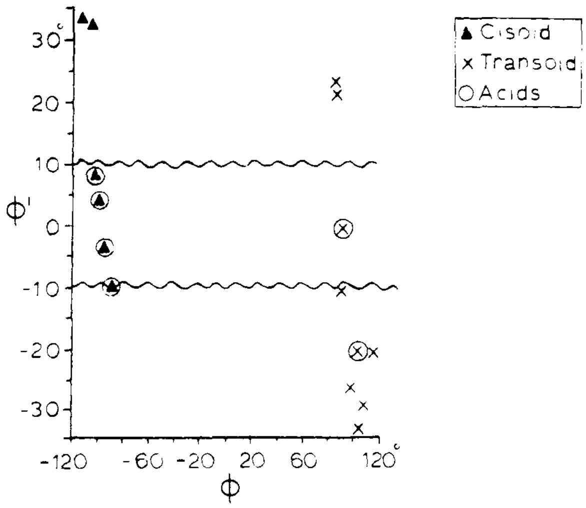

Figure 3. A plot of the diphenyl ether torsion angles ϕ and ϕ' for 15 thyroactive crystal structures studied. Those structures which are deamino compounds are circled. The triangles are cisoid and the × signs are transoid.

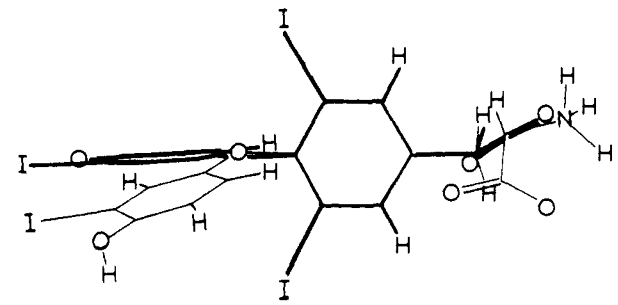

Figure 4. A superposition of T_3AA (dark) with T_3 (light) illustrating the skewed and twist–skewed conformations.

T_3 and its methyl ester (Table II). The two thyroacetic acid metabolites also have diphenyl ether conformations which are skewed ($\phi = \pm 90°$, $\phi' = 0°$), in contrast to the thyroxine derivatives which deviate from this geometry. On the basis of these structures, it would appear that the presence of an acetic acid side chain rather than an amino acid side chain stabilizes a skewed ring conformation.

In Figure 3, which shows all thyroactive crystal structures reported to date,[9,10] those conformational parameters for deamino acid structures are circled. With only one exception, these values fall within a 10° range of the skewed conformation ($\phi = \pm 90°$, $\phi' = 0°$). Thus, the observations made for the acetic acid metabolites can be extended to include all deamino acids in their preference for a skewed conformation. It would then appear that the absence of the α-amino nitrogen, rather than its presence, controls this conformation.

The amino acid structures are then paired on either side of this skewed range and, with few exceptions, into $\pm\phi'$ groups for the cisoid and transoid sets, respectively. The average amino acid conformation[10] then can be described as "twist–skewed" ($\phi = 108°$, $\phi' = -28°$; $\phi = -108°$, $\phi' = 28°$). Further subsets in the number of preferred conformers among the amino acids are indicated from other correlations among these parameters. These observations suggest that there are at least four preferred conformers; transoid and cisoid skewed and transoid and cisoid twist–skewed forms. The significant differences between the skewed and the twist–skewed conformers are illustrated in Figure 4.

These results should contribute to the improved interpretation of solution spectral data on thyroid hormones where the possibility of unrestricted flexibility is difficult

Table II. Conformational Parameters (Degrees) for Thyroxine Analogues

Structure	ϕ[a]	ϕ'[b]	χ^2[c]	χ^1[d]	ψ^1[e]	ψ^2[f]	Ref
3,5,3'-Triiodothyroacetic acid[g]	92	−1	78			33	12
3,5,3',5'-Tetraiodothyroacetic acid[g]	−95	−4	129			−8	
3,5,3'-Triiodo-L-thyronine	116	−21	76	−164	8		6
3,5,3'-Triiodo-L-thyronine methyl ester	−108	33	121	−54	8		7
3,5,3'-Triiodo-L-thyronine hydrochloride	90	−11	98	56	8		13
3,5,3',5'-Tetraiodo-L-thyronine (1)[g]	108	−30	152	−170	−41		14
(2)	−112	33	87	−160	49		
3,5,3',5'-Tetraiodo-L-thyronine hydrochloride	105	−34	98	67	−39		13

[a] ϕ = C(5)-C(4)-O(41)-C(1'). [b] ϕ' = C(4)-O(41)-C(1')-C(6'). [c] χ^2 = C(2)-C(1)-C(7)-C(8). [d] χ^1 = C(1)-C(8)-C(8)-N(8). [e] ψ^1 = N(8)-C(8)-C(9)-O(10). [f] ψ^2 = C(1)-C(7)-C(8)-O(8). [g] NDEA = *N*-diethanolamine (1:1) complex.

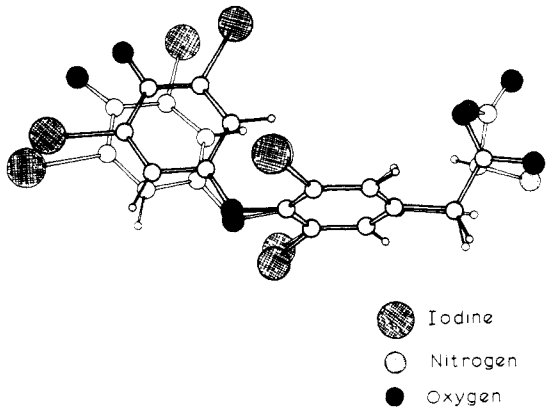

Figure 5. A superposition of T_4 (dark) with T_4AA illustrating the maximum degree of overlap between these crystallographically observed conformations. The dark molecule is above the light one.

to distinguish from the presence of mixed populations of principal conformers in solution. These data could also provide more accurate parameters for the calculation of conformational energies.

Furthermore, when the crystal structures of thyroid binding proteins are determined, these data on the substrate conformational flexibility will be vital to the accurate interpretation of molecular details at the active site.

The observation that T_3AA binds 16 times more strongly to plasma proteins[3a] than does T_3, but much less than T_3 to other binding proteins,[3b,4] suggests that in some instances the presence of the amino group may be inhibitory to binding. It could also be that the skewed conformation of the diphenyl rings characteristic of the acetic acid derivatives is preferred in plasma protein binding, whereas the "twist-skewed" form is optimal for binding to other proteins. This also suggests that specificity of binding to different proteins by the thyroid hormones and their metabolites may be associated with a change in the amino acid portion of the molecule.

The maximum degree of overlap between the thyroacetic acid structures and their respective parent hormones is achieved when the thyroid hormone has a conformation in which the α-amino function is fully extended with respect to the inner phenyl ring (χ^1 = 180°). In this conformation, shown in Figure 5, one of the carboxylic oxygens [O(9)] comes nearest to occupying the same space as the nitrogen. This conformation has been shown to be an energetically favorable conformation for aromatic amino acids.[11]

A pattern of conformational isomerism in the thyroid hormones continues to develop. The 3'-iodine in T_3 has been observed in proximal and distal conformations and the latter has been demonstrated to be the active form. Crystallographic observations first brought to light the dramatic distinction between transoid and cisoid forms, and structural data presently available suggest an equal distribution between these conformers in various environments. Now for the first time a pattern is emerging in the diphenyl ether conformations which are seen to be either skewed (ϕ,ϕ'; ±90,0°) or twist-skewed (ϕ,ϕ'; ±108, ∓28°), which may be linked to differences in the binding affinities and hormone action.

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Supplementary Material Available: Positional and anisotropic thermal parameters, bond lengths and angles, calculated structure factors, and packing diagrams (24 pages). Ordering information is given on any current masthead page.

References and Notes

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(15) In order to develop a consistent numbering scheme for all thyroactive compounds, a standard orientation was chosen such that the C^{α} atom was viewed above the inner phenyl ring plane. The inner phenyl ring was then numbered clockwise and the outer phenyl ring is numbered in such a manner that the C(2′) atom is always distal to the inner ring. In this manner, all the compounds are self-consistent and correlations between ϕ and ϕ' can be detected.

Structure–Activity Studies on Hallucinogenic Amphetamines Using Molecular Connectivity

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A series of ring-substituted hallucinogenic amphetamines has been analyzed using molecular connectivity. A correlating equation has been found between potency and connectivity terms. The equation permits an interpretation of SAR. The equation is capable of predicting potency for amphetamines not in the list and mescalines and tryptamines.

The contributions of Shulgin and his colleagues have afforded an opportunity to study structure–activity relationships (SAR) among a fairly large list of hallucinogenic agents.[1] Specifically, the availability of over a score of ring-substituted amphetamines with comparative hallucinogenic potencies makes it possible to examine the structural features contributing to the activity.

Snyder and Merril were the first to use these data in a study employing semiempirical quantum mechanical calculations.[2] Their Hückel MO calculations showed some relationship between the energy of the highest occupied MO, E (HOMO), and the potency, established by Shulgin, among limited sets of amphetamine and tryptamine derivatives. Kang and Green calculated the same index using the INDO MO method for a set of 14 substituted amphetamines.[3] They found a modest correlation with potency.

These studies gave an indication that the substitution pattern on the amphetamine ring may influence the interaction with a receptor feature through forces of the van der Waals type. This has prompted us to analyze the interaction energies of 17 derivatives with model receptor molecules.[4] The correlation found, $r = 0.85$, was encouraging.

Barfknecht has pursued a different approach by attempting to relate partition coefficients of neutral amphetamines with potency.[5] The correlation with activity was modest. It is not certain how partition coefficients can be interpreted in terms of molecular structure of the amphetamine analogues.

In recent studies of biological SAR,[6-9] we have analyzed molecular structure in terms of the number and kinds of atoms, bonding type, and adjacency environment. This method, having its roots in topology, is called molecular connectivity.[9] The theory, formalism, and applications have been described in one source.[9] A condensed description of molecular connectivity calculations is given in Appendix I.

It is well known that measured property values vary with change in molecular structure. What is meant by change in structure? Structural change usually includes variation in the number and type of atoms, branching, cyclization, and change in bond types. Such properties as boiling point and molar refraction of normal alkanes and normal primary alcohols illustrate structural influence on properties. There is a linear relationship between these properties and the number of carbon atoms for these straight-chain molecules. The same trend is observed in homologous series of molecules which produce many interesting biological phenomena.

In these cases structural information necessary to establish structure–activity relationships is simply the number of carbon atoms. At the present time such relationships cannot be developed de novo from quantum mechanics and thermodynamics so that these properties could be predicted directly from the number of carbon atoms alone. However, a relating equation can be established by standard regression analysis.

Now consider branching in alkanes or alcohols. The information contained only in the number of carbon atoms is inadequate for establishing close relationships to properties. It is well known to organic chemists that chain branching within a set of isomers leads to lower boiling points. How may such a structural characterization, in this case branching, be represented in quantitative terms suitable for establishing useful relationships? The number of carbon atoms is easily quantitated, but how may branching be quantitated?

Beyond branching one encounters the occurrence of heteroatoms, multiple bonding, and cyclization, features which are not readily quantitated in a form suitable for SAR. Molecular connectivity attempts to describe quantitatively these kinds of structural features at the same level of information as an atom count, i.e., a numerical value which can be determined unambiguously for a given molecule and which is also transferrable from study to study.

Among isomers the number of atoms and bonds remains constant; hence, these numbers are inadequate for structural description. Molecular connectivity begins with this branching pattern by depicting the molecular structure as the familiar skeleton formula. Based on this molecular