Thyroid Hormone Stereochemistry. III. Molecular Structure of Triiodothyropropionic Acid Ethyl Ester[1]

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The three-dimensional structure of triiodothyropropionic acid ethyl ester has been determined as part of an investigation of the stereochemistry of the thyroid hormones. The compound crystallizes in the monoclinic space group $P2_1/c$, with cell dimensions $a = 14.60$, $b = 8.843$, $c = 16.70$ Å, $\beta = 111°27'$; $Z =$ four molecules per cell. The structure was determined by direct centrosymmetric phasing procedures to locate the iodine atoms and phasing on the iodines to find the light atoms. Refinement was by anisotropic full-matrix least squares to a final discrepancy value $R = 0.038$.

The two phenyl rings in the molecule are skewed with respect to each other and are not far from being mutually perpendicular, with angles of 88 and 10° between the plane of the inter-ring ether linkage and the planes of the diiodo-ring and the monoiodo-ring, respectively. The conformation is such that the 3′-iodine atom is *proximal* to the diiodo-ring, similar to the molecular conformation found in the crystal structure of triiodo-L-thyronine hydrochloride.

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On détermine la structure tridimensionnelle de l'ester éthylique de l'acide triiodothyropropionique comme une partie de l'étude stéréochimique des hormones thyroides. Le composé cristallise dans le système monoclinique, groupe d'espace $P2_1/c$ avec les dimensions de la maille $a = 14.60$, $b = 8.843$, $c = 16.70$ Å, $\beta = 111°27'$; $Z = 4$ molécules par maille. On détermine la structure par des procédés directs de phasage centrosymétrique pour localiser les atomes d'iode; on phase les iodes pour trouver les atomes légers. On affine par moindres carrés à matrice entière anisotrope à une valeur finale de $R = 0.038$.

Les deux noyaux phényles dans la molécule sont gauches l'un par rapport à l'autre, et ne sont pas loin d'être perpendiculaires avec les angles de 88 et 10° entre d'une part le plan de lien éther entre les noyaux et, d'autre part, les plans respectifs du noyau diiodé et du noyau monoiodé. La conformation est telle que l'atome iodo-3′ est le plus proche du noyau diiodé: elle est similaire à la conformation moléculaire trouvée dans la structure cristalline du hydrochlorure triiodo L-thyronine. [Traduit par le journal]

Introduction

Triiodothyropropionic acid (T_3P) (β-[4-(3′-iodo-4′-hydroxyphenoxy)-3,5-diiodophenyl]propionic acid) is chemically very similar to triiodothyronine (T_3), the most potent naturally-occurring thyroid hormone; the two molecules differ in chemical structure only in that T_3P lacks an α-amino group. In various tests of thyromimetic action T_3P exhibits from 2–20% of the hormonal potency of T_3 and from 10–60% of the activity of thyroxine, depending on the biological function being measured (1). Indeed, in one test, the determination of the minimal dose of a compound which would promote metamorphosis of the tadpole (*Rana pipiens*), T_3P was 60 times as active as T_3 and 300 times more active than thyroxine (1). It has also been found (1) that T_3P administered to myxedematous patients mimicked T_3 in lowering serum cholesterol levels but had little effect on the basal metabolism rate; these results indicate that certain physiologic effects of thyroid hormones can be dissociated by alteration of molecular structure. The thyromimetic action of T_3P has led to its marketing in Europe as a thyroid supplement and cholesteropenic agent.

We have recently determined the three-dimensional structure of triiodo-L-thyronine (2) and

[1]For Part II of this series see ref. 8.

found that the molecular conformation is such that the β-ring 3′-iodine is situated *proximal* to the diiodotyrosyl (α) ring, rather than *distal* as was concluded from chemical structure – biological activity tests (3). Though molecular orbital calculations (2) gave additional support for the greater stability of the 3′-iodine *proximal* conformation than the *distal* possibility in T_3, it is highly desirable to seek other structural data of T_3 analogs to clarify this conformational question. The close chemical similarity of T_3P to T_3 and the fact that it also possesses some degree of thyromimetic activity make it the obvious and ideal choice for structural elucidation and conformational comparison with T_3. Thus we have determined the three-dimensional molecular structure of triiodothyropropionic acid ethyl ester in order to see if the 3′-iodine *proximal* conformation found for T_3 is the generally favored one for this type of thyromimetic agents and to see if differing stereochemical features can be invoked to clarify the differing spectra of biological actions of these two hormones.

Experimental

Triiodothyropropionic acid (whitish powder) was dissolved in an ethanol–HCl solution and thin colorless diamond shaped plates (later shown by the structure determination to be the ethyl ester of T_3P) were obtained by solvent evaporation. The unit cell dimensions were determined from diffractometer measurements and crystal data are as follows: ethyl triiodothyropropionate, $C_{17}H_{15}I_3O_4$, mol. wt. 664.02, monoclinic, $a = 14.60 \pm 0.01$, $b = 8.843 \pm 0.005$, $c = 16.70 \pm 0.01$ Å, $\beta = 111° 27' \pm 10'$, space group $P2_1/c$. The density, calculated on the basis of four molecules per unit cell is 2.199 g cm^{-3}. Absorption coefficient, μ (for Mo Kα radiation) is 47.5 cm^{-1}.

Intensity data were collected on an automated four-circle diffractometer (Zr-filtered Mo radiation) and all independent reflections with 2θ (MoKα) < 48° (corresponding to a minimum interplanar spacing of 0.87 Å) were measured. The moving crystal – moving counter technique was employed (2θ scan) with stationary counts for background radiation on each side of the reflection. A total of 3152 reflections was measured, of which 2488 had intensity greater than $2\sigma_c$, where $\sigma_c = (N_{B1} + N_{PK} + N_{B2})^{1/2}$, N_{B1} and N_{B2} are the background counts, and N_{PK} is the scan count. Those reflections with intensities less than $2\sigma_c$ were classified "unobserved" and were not used in structure refinement. The crystal used for data collection had dimensions approximately 0.1 × 0.2 × 0.9 mm, and absorption corrections were applied using a modified version of a computer program by Coppens *et al.* (4). Lorentz and polarization factors were applied and structure amplitudes $|F|$ and normalized structure amplitudes $|E|$ were derived.

Structure Determination

The symbolic addition procedure was used to determine the phases of the reflections. Three reflections were assigned phases of 0°, three more were assigned symbolic phases, and the Σ2 formula was iteratively applied. Phases were ultimately determined for 391 reflections with $|E| \geq 1.4$ and these were used to calculate a three-dimensional E map. It had been noted early in the structure determination that almost all of the planes with high $|E|$ values had indices of the form l = even (only 2 of 86 planes with $|E| \geq 2.2$ had l = odd and similarly only 22 of 184 planes with $|E| \geq 1.9$), which indicates that the iodines in the molecule of T_3P (ethyl ester) are situated at rather special positions in the cell with respect to their y coordinates. This was confirmed by the E map: there were four major peaks on the map and all had y coordinates close to 0, 1/2, or 3/4. In addition, one of the peaks was at $x = 0.25$ and two of the others were related to each other by a pseudo mirror plane at $x = 0.25$. One of these mirror-related peaks was chosen along with the other two independent peaks as the positions of the three iodines, structure factors were calculated and a difference-Fourier map was computed. All of the carbon and oxygen atoms of the T_3P molecule were clearly revealed and additional density in the map showed that the compound was the ethyl ester of triiodothyropropionic acid, esterification apparently having taken place during crystallization from HCl–ethanol.

Structure Refinement

The atomic coordinates and thermal parameters were refined by full-matrix least squares. The function minimized was $\Sigma w (|F_o| - |F_c|)^2$ with weights $w = (1/\sigma_F)^2$ and the atomic scattering factors were taken from the International Tables for X-Ray Crystallography (5).[2] Two cycles of refinement with isotropic thermal parameters resulted in a discrepancy index $R = 0.099$ and two anisotropic cycles lowered R to 0.044. The hydrogen atoms were located from difference Fourier maps and three cycles of refinement, allowing everything to vary except the hydrogen thermal parameters (held fixed at the values of the atoms to which the hydrogens are bonded) resulted in a final R of 0.038. The atomic fractional coordinates and thermal parameters are given in Table 1, in which the U_{ij} are coefficients in the expression exp $[-2\pi^2 (h^2U_{11}a^{*2} + ... + 2klU_{23}b^*c^*)]$. The table of final observed and calculated structure factors may be found in the Depository of Unpublished Data.[3] Coordinates for the hydrogen atoms are given in Table 2.

Discussion

A stereoscopic drawing of ethyl triiodothyropropionate illustrating both the molecular conformation and the relative magnitudes and

[2]The scattering curve for iodine was not corrected for anomalous dispersion.

[3]Tables of final observed and calculated structure factors for T_3P are available, at a nominal charge, from the Depository of Unpublished Data, National Science Library, National Research Council of Canada, Ottawa, Canada, K1A 0S2.

TABLE 1. Fractional atomic coordinates and anisotropic thermal parameters ($\times 10^5$)

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
I(3′)	0.2491	0.0864	0.3744	726	570	719	8	332	104
I(5)	0.4454	0.7547	0.4804	634	628	646	87	323	−30
I(3)	0.3369	0.5108	0.1205	756	775	576	−41	204	−1
O(1)	0.0576	0.2650	0.3520	564	845	1181	−43	520	−88
O(2)	0.3167	0.6604	0.2884	455	473	716	160	229	139
O(3)	0.9091	0.3960	0.3930	593	1186	1119	275	533	320
O(4)	0.9063	0.2894	0.5127	649	949	758	258	272	61
C(1′)	0.2538	0.5573	0.3064	400	500	442	78	178	−29
C(2′)	0.2813	0.4079	0.3292	373	569	502	95	162	28
C(3′)	0.2123	0.3144	0.3436	417	468	452	29	153	−24
C(4′)	0.1209	0.3661	0.3358	402	606	522	5	241	−66
C(5′)	0.0976	0.5129	0.3152	393	454	762	29	336	−117
C(6′)	0.1660	0.6094	0.3003	524	794	683	125	227	−26
C(1)	0.6112	0.5526	0.3390	549	675	781	105	340	150
C(2)	0.5384	0.5214	0.2589	600	608	611	105	288	44
C(3)	0.4431	0.5550	0.2434	392	476	549	9	175	66
C(4)	0.4138	0.6204	0.3060	425	430	705	82	366	94
C(5)	0.4846	0.6495	0.3856	539	320	594	13	268	23
C(6)	0.5820	0.6155	0.4006	542	583	499	88	207	52
C(7)	0.7178	0.5148	0.3552	542	960	906	145	456	126
C(8)	0.7698	0.4371	0.4362	543	969	716	124	336	88
C(9)	0.8689	0.3750	0.4426	473	672	802	86	324	29
C(10)	1.0029	0.2248	0.5282	806	976	1102	348	332	141
C(11)	1.0352	0.1486	0.6117	1207	1482	934	668	282	233
				Standard deviations					
I	0.0001	0.0001	0.0000	4	5	4	3	3	3
O	0.0005	0.0009	0.0006	43	59	62	40	43	42
C (ring)	0.0007	0.0011	0.0007	56	55	55	43	43	42
C (7–10)	0.0009	0.0017	0.0008	61	85	73	61	59	63
C (11)	0.0025	0.0025	0.0018	138	163	95	140	93	116

TABLE 2. Hydrogen atom fractional coordinates

Atom	x	y	z	Atom	x	y	z
H(O)	0.019	0.296	0.360	H(8)	0.723	0.310	0.451
H(2′)	0.339	0.392	0.335	H(8*a*)	0.766	0.486	0.476
H(5′)	0.028	0.538	0.316	H(10)	0.988	0.124	0.493
H(6′)	0.159	0.689	0.295	H(10*a*)	1.052	0.258	0.517
H(2)	0.559	0.503	0.226	H(11)	0.962	0.023	0.561
H(6)	0.624	0.629	0.453	H(11*a*)	1.091	0.084	0.613
H(7)	0.728	0.434	0.325	H(11*b*)	1.002	0.100	0.627
H(7*a*)	0.760	0.617	0.345	Standard deviations	0.007–013	0.010–020	0.006–010

shapes of the atomic thermal ellipsoids is shown in Fig. 1. The conformation of the molecule is such that the 3′-iodine atom is *proximal* to the α-diiodo ring and the uniodinated 5′ position is *distal*, identical to the conformation observed in the crystal structure of triiodo-L-thyronine (2). The aromatic rings are planar (Table 3) and are close to being completely skewed with respect to each other and mutually perpendicular. The angles between normals to the plane of the inter-ring ether linkage and the planes of the α-diiodo and β-monoiodo rings are ± 88 and $\mp 10°$, respectively; these values compare closely with the values of $+86$ and $-13°$ for the related angles in triiodo-L-thyronine and are also similar to angles $+86$ and $+19°$ found in 3,5-diiodo-L-thyronine when it is complexed with *N*-methylacetamide (6). A plus value of the angle indicates rotation of the phenyl ring away from coplanarity with the plane of the inter-ring ether linkage in an anticlockwise direction when viewed along the (phenyl)C—O(ether) bond in the direction from

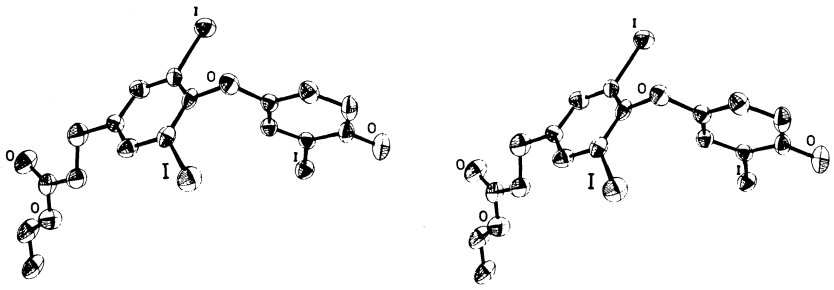

FIG. 1. Stereoscopic drawing of ethyl triiodothyropropionate showing the molecular conformation and the relative sizes and shapes of the thermal motion ellipsoids.

TABLE 3. Deviations of atoms from least-square planes

Plane 1		*Plane 2*		*Plane 3*	
Atom	Δ (Å)	Atom	Δ (Å)	Atom	Δ (Å)
C(1)	0.007	C(1′)	0.008	O(3)	0.002
C(2)	−0.006	C(2′)	−0.004	O(4)	0.001
C(3)	0.000	C(3′)	−0.006	C(8)	0.001
C(4)	0.006	C(4′)	0.011	C(9)	−0.004
C(5)	−0.004	C(5′)	−0.006	C(7)[a]	0.189
C(6)	−0.002	C(6′)	−0.003	C(10)[a]	−0.010
C(7)[a]	0.003	O(1)[a]	0.000	C(11)[a]	−0.258
O(2)[a]	0.072	O(2)[a]	0.045		
I(3)[a]	0.035	I(3′)[a]	0.035		
I(5)[a]	0.077				

[a]These atoms not used in calculating the plane.

C to O. The angles for T_3P have dual signs because the $P2_1/c$ space group contains a glide plane which means that both conformers must exist in the crystal. Thus the conformation of the diphenyl ether parts of these three molecules are remarkably similar. The conformation of this part of the L-thyroxine molecule (7) differs somewhat from these, with greater deviations of the two rings from being perpendicular to and coplanar with the inter-ring ether plane (angles of +101 and −34°).

The conformation of the propionic acid chain in T_3P differs markedly from that of the alanine chains in L-thyroxine, triiodo-L-thyronine, and L-thyronine (8). In these latter three molecules the alanine conformations are virtually identical, whereas in T_3P, in which the propionic acid chain differs from alanine only in that it lacks the α-amino group of alanine, the conformation about the C(α)—C(β) bond is different from that occurring in the thyronine derivatives; the side chain in T_3P is more extended and lies slightly more in a direction "proximal" to the β-ring, rather than "distal" to it as is the case with the alanine chains (see Fig. 1 and related figures for the thyronine derivatives in refs. 2, 7, and 8). The atoms comprising the carboxyl grouping lie on a plane (Table 3).

Bond lengths and angles in the T_3P molecule are shown in Fig. 2. Because the iodine atoms dominate the X-ray scattering the estimated standard deviations for light atom bond distances and angles are rather high (0.015–0.022 Å, except for C(10)—C(11) which is 0.035 Å, and 0.7–1.8°) but the values are all near normal and require no special comment. The iodine–carbon bond lengths average 2.10 Å, in good agreement with average values of 2.11 Å in triiodo-L-thyronine and L-thyroxine, and 2.10 Å in 3,5-diiodo-L-thyronine. This is longer than the normal carbon (aromatic) – iodine distance of 2.05 Å and may explain why the iodinated thyronine-type compounds often exhibit tendencies toward decomposition when irradiated with X-rays, with apparent liberation of iodine. The angle at the inter-ring ether linkage is 119°, similar to what has been found for other diphenyl ether compounds. Bond distances and angles involving

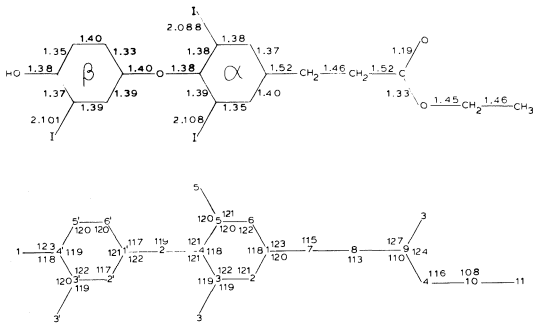

FIG. 2. Bond lengths (Å) and angles (°) in ethyl triiodothyropropionate.

hydrogen atoms are omitted from Fig. 2: the inaccuracies in their values are too large to merit their inclusion.

The hydroxyl group is the only hydrogen donor in the molecule for hydrogen bonding and it takes part in an intermolecular contact with the carbonyl oxygen in the molecule one unit cell away in the x direction; the hydrogen...carbonyl oxygen distance is 2.1 Å (oxygen–oxygen distance = 2.76 Å) and the O—H...O angle is 176°. There is also a network of short intermolecular iodine...iodine distances: every I(3′) at (x,y,z) has an intermolecular contact of 4.03 Å with an I(5) in a related molecule at $(x, 1/2-y, 1/2+z)$, and one of 3.93 Å with an I(3) in a molecule at $(x, y-1, z)$, and these latter two atoms are separated by 3.78 Å. These intermolecular separations are significantly shorter than the normal I...I van der Waals separation of 4.30 Å given by Pauling (9) (though only slightly shorter than the 4.08 Å value of Bondi (10)) and indicate some degree of charge transfer bonding may be taking place among the iodines, aiding in stabilizing the crystal structure.

The elucidation of the crystal and molecular structures of ethyl 3,5,3′-triiodothyropropionate has shown that the molecular conformation is such that the 3′-iodine is situated *proximal* to the α-ring, identical to the results found in the structure determination of triiodo-L-thyronine. Thus the structural evidence obtained so far consistently indicates this conformation to be an energetically favored one, whereas the indications of Jorgenson *et al.* (3), from biological activity studies of thyroid hormone analogs, are that the 3′-iodine *distal* conformation is biologically more active. Further clarification of these contradictory results must await structural studies of other thyromimetic agents and of the analogs used in the biological activity studies; such investigations are now in progress in these laboratories.[4,5]

The conformation of the propionate chain of T_3P is quite different from the alanine conformation found in L-thyroxine, triiodo-L-thyronine, and L-thyronine ethyl ester; and since the diphenyl ether parts of T_3P and triiodo-L-thyronine are conformationally virtually identical, the differences in biological potency of the two hormones undoubtedly result from these differing side chain structures. The essential differences are (*a*) absence of the amine functional group in T_3P and (*b*) the change in conformation about the β-carbon atom of the chain.

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[4]Since preparation of this work for publication a note has appeared (see ref. 11) describing the crystal structure of 3,5,3′-triiodothyroacetic acid *N*-diethanolamine (1:1) complex, in which the 3′-iodine was found to be oriented distal to the α-ring. The authors also refer to unpublished data for other structures having distal orientations for 3′-iodines. At the same time we have described the results of a structural investigation of 3′-isopropyl-3,5-diiodo-L-thyronine, the most potent known thyromimetic agent (see ref. 12) in which the conformation is such that the 3′-isopropyl group is proximal to the α-ring. Our crystals were prepared from acidic alcoholic solutions, while Cody and Duax (11) appear to have had large excesses of organic reagents present in their crystallization media. These results indicate that crystallization conditions may play a significant role in influencing the conformation of these asymmetric thyronine derivatives, and will undoubtedly provoke much interesting work on structural and biological aspects of thyroid hormones and analogs under different environmental conditions.

[5]We have recently performed high-resolution n.m.r. studies of T_3P in solutions similar in composition to those trom which the crystalline proximally- and distally-orienfed T_3- and T_3-like analogs were obtained. The spectra are consistent with there being two resolvable conformers in acidic media, the proximal conformer dominating, and only one conformer (or, more probably, a time-averaged conformation) in neutral solution (see ref. 13).

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