THYROID HORMONE STRUCTURE: MOLECULAR CONFORMATION OF 3'-ISOPROPYL-3,5-DIIODO-L-THYRONINE, THE MOST POTENT KNOWN THYROMIMETIC AGENT

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Received March 26,1973

SUMMARY. The three-dimensional structure of the potent thyromimetic agent 3'-isopropyl- 3,5-diiodo-L-thyronine (iPr-T_2) has been established by x-ray diffraction of single crystals of iPr-T_2 hydrochloride. The molecular conformation is such that the β-ring 3'-isopropyl group is oriented in space proximal to the 3,5-diiodotyrosine α-ring, similar to the conformation adopted in the crystal structure of 3,5,3'-triiodo-L-thyronine.

INTRODUCTION

Much experimentation has been carried out in recent years with the aim of correlating chemical and conformational structure with biological activity for the thyroid hormones. The molecular asymmetry of the β-ring in 3,5,3'-triiodo-L-thyronine (T_3) (Ib) appears to play an important role: T_3 demonstrates 3-4 times the activity of L-thyroxine (T_4) which has a symmetric β-ring (Ia). Although the 3' and 5' positions on the β-ring are chemically equivalent, the expected 120° angle at the ether oxygen atom produces conformational variability in T_3, and since the two iodinated phenyl rings are most likely to be positioned in roughly mutually perpendicular planes this results in two conformations for T_3 which are more likely than any others: one with the 3'-iodine oriented

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distal to the α-ring, and the other with the 3'-iodine proximal. Jorgenson et al (1,2) synthesized and tested analogues of T_3 in which rotation of the β-ring was sterically hindered and found that the analogue with the 3'-substituent "fixed" in the distal orientation demonstrated much greater hormonal activity than the 3'-proximal isomer. However, the recent determination of the three-dimensional structure of T_3(3) showed it to have the 3'-iodine proximal conformation in the crystal, and energy calculations indicated this molecular conformation to be the more stable one. Further structural studies were clearly needed, and we determined the crystal structure of 3,5,3'-triiodothyropropionic acid (T_3P), a thyromimetic agent which differs chemically from T_3 only in that T_3P lacks the α-amino group of T_3. The results were similar to those of T_3: the T_3P conformation is such that the 3'-iodine is also oriented proximal to the α-ring (4).

In this study we present the three-dimensional structure of 3'-isopropyl-3,5-diiodo-L-thyronine (iPr-T_2), (Ic), in which the 3'-iodine of T_3 is replaced by an isopropyl group. This compound is of great interest because it is the most potent of all thyroid agents, having hormonal activity 50-100% greater than T_3 (2,5,6,7). In addition, with the isopropyl group replacing the 3'-iodine we eliminate the possibility of the proximal conformation being stabilized by charge-transfer interactions between 3'-iodine 5-d orbitals and α-ring π electrons or other electron donors. If interactions of this sort play a decisive role in stabilizing the proximal positioning of the 3'-iodine in the crystal structure, the substitution of the isopropyl group should facilitate adoption of the distal conformation.

The crystals of iPr-T_2 are unstable to x-irradiation, and accurate refinement of structural parameters must await collection of better data from new and better crystals. Unfortunately this is not easy to achieve, and because of the interest in the stereochemical features of these hormones we feel it desirable to publish our results rapidly in the present form.

METHODS

3'-isopropyl-3,5-diiodo-L-thyronine was dissolved in warm 1N.HCl and slow solvent evaporation yielded clumps of tiny colorless needle-shaped crystals of iPr-T_2 hydrochloride. The crystals are monoclinic, with cell dimensions $\underline{a}$ = 30.43, $\underline{b}$ = 5.31, $\underline{c}$ = 17.17 Å, β = 117.8°, space group C2 with 4 molecules of iPr-T_2 HCl (and, as revealed by subsequent structure solution, 12 molecules of H_2O) per unit cell. Intensities of 1315 independent x-ray reflections to 2θ = 40° for MoKα radiation (minimum interplanar spacing of 1.04 Å) were measured on an automated four-circle diffractometer, and 793 of these had intensities greater than twice the standard deviations of their measurements. The intensities of reflections monitored as standards fell appreciably during the data collection and the crystal became yellowish, indicating decomposition of the crystal during x-ray exposure, probably with liberation of iodine. A linear correction factor was made for the fall-off of intensities with time and structure amplitudes were derived in the usual manner.

Positions of the two iodine atoms and the chloride ion were obtained by analysis of the Patterson function: their coordinates are very similar to those of the corresponding atoms in the crystal of T_3. The positions of the carbon, nitrogen, and oxygen atoms were found from two cycles of weighted Fourier summations, utilizing initially phases based on the iodines and chlorine. Three water molecules were also found to be present for each molecule of iPr-T_2. The atomic positions and thermal parameters (anisotropic for iodine and chlorine, isotropic for the others) were refined by full-matrix least squares (discrepancy index $\underline{R}$ = 0.096) and a difference Fourier was calculated. Fifteen of the twenty hydrogen atoms on the iPr-T_2 molecule were visible on the difference map, and with these included in the structure factor calculation but not refined, the final $\underline{R}$ = 0.091.

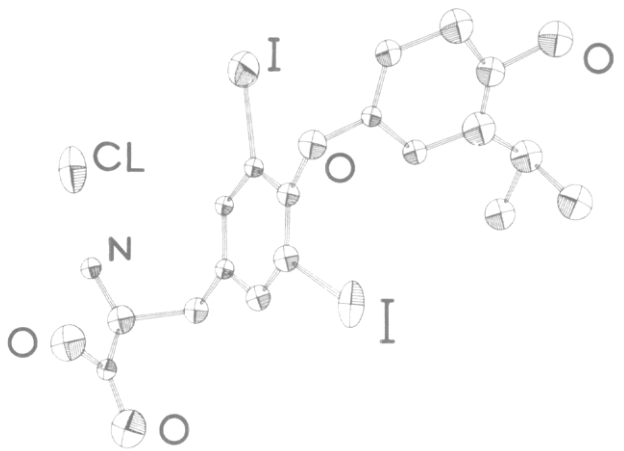
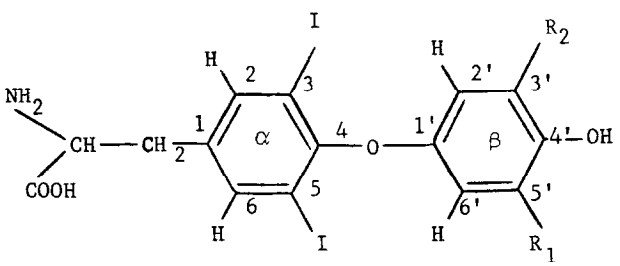


Fig. 1. Molecular conformation of 3'-isopropyl-3,5-diiodo-L-thyronine hydrochloride.



Ia: $R_1 = I = R_2$
Ib: $R_1 = H$, $R_2 = I$
Ic: $R_1 = H$, $R_2 = CH(CH_3)_2$

DISCUSSION

Figure 1 is a perspective drawing of the molecular conformation of 3'-isopropyl-3,5-diiodo-L-thyronine hydrochloride. The conformation is such that the β-ring is oriented in space with its 3'- isopropyl group positioned proximal to the α-ring, similar to the 3'-iodine position in the structures of T_3(3) and T_3P (4). The planes of the phenyl rings approach mutual perpendicularity, the angles between the α- and β-rings and the plane of the inter-ring ether linkage being 86° and 21° respectively (vs. 86° and 13° in T_3, 88° and 10° in T_3P, and 86° and 19° in 3,5-diiodo-L-thyronine N-methyl acetamide (8)). The angle at the inter-ring

oxygen measures 125°, larger than the 119-120° values in the other compounds cited but the difference is not significant as the rather poor intensity data and the domination of x-ray scattering by the iodine and chlorine atoms contribute to large standard deviations for light-atom parameters. The iodine-carbon bond lengths average 2.12 Å, similar to the values of 2.10-2.11 Å observed in the other iodinated-thyronines.

The conformation of the alanine chain of iPr-T_2 is very similar to the alanine parts of T_3, L-thyroxine (9), and L-thyronine (10), indicating a constancy of conformational characteristics in the amino acid parts of thyronine derivatives.

The fact that iPr-T_2 is the most potent thyromimetic agent known indicates that it is the steric properties of the β-ring 3'-substituent which are important, not the chemical (e.g. electron-donating or accepting) properties. Therefore the interaction of the 3'-iodine of T_3 with its biological receptor is likely to be of a steric or hydrophobic nature, and not of the charge-transfer type. T_3 could conceivably take part in inter-molecular charge-transfer attractions via the 3'-iodine in the crystalline state, thus lending an extra degree of stability to one conformation or the other (though no evidence of this was actually found in the T_3 crystal structure); iPr-T_2, on the other hand, has a relatively inert group at the 3'-position and its conformation cannot be further stabilized inter-molecularly through this substituent by other than normal van der Waals interactions. Because the iPr-T_2 molecular conformation is so similar in all respects to that adopted by T_3, this presents persuasive evidence that the 3'-substituent proximal positioning is not an artifact of the crystal structure, but is indeed the inherently more stable one.

ACKNOWLEDGMENTS

We thank Smith Kline and French Canada Ltd. for a gift of 3'-isopropyl-3,5-diiodo-L-thyronine. Support was from the Medical Research

Council of Canada, and the USPHS through the University of Washington Medical School general research support grant.

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