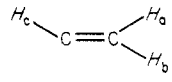
in several days at −5 °C. The collected product weighed 500 mg: mp >170 °C dec; NMR (Me_2SO-d_6) δ 1.18 (d, CH_3CH), 3.77 (s, OCH_3), 5.38 (2 d, i), 6.78 (2 d, v, $J_{c,a}$ = 10 Hz, $J_{c,b}$ = 18 Hz). Anal.



v

($C_{18}H_{25}N_3O$·0.9H_3PO_4) C, H, N. A sample of this salt was converted back to the free base **7b**; high resolution mass spectrum *m/e* required for $C_{18}H_{25}N_3O$, 299.1997; observed, 299.1995.

2-(β-Bromoethyl)-8-[[6′-(Diethylamino)hexyl]amino]-6-methoxy-4-methylquinoline (8b) Hydrobromide. The 8-aminoquinoline **6** (3 g, 0.014 mol) was mixed with a few drops of DMF and heated at 75 °C while a solution of 7 g (0.022 mol) of 6-(diethylamino)hexyl bromide hydrobromide in 4 mL of DMF and 0.20 mL of water was added with stirring in 6 h. The pasty mixture was cooled overnight and diluted with some ethanol and THF, and the solid was separated by filtration to yield 1.3 g of yellow powder, which turned brown upon storage at room temperature. The melting point, 200–205 °C, did not change upon one precipitation of the product from ethanol–THF: yield of yellow-brown material 1 g (13%); NMR (Me_2SO-d_6) showed no signals which could be assigned to vinyl protons; UV (CH_3OH) λ_{max} 270 nm, 290 (shoulder), 351. For comparison, compound **8a**: UV (CH_3OH) λ_{max} 270 nm, 290, 352; 8-amino-6-methoxy-4-methyl-2-vinylquinoline hydrobromide: UV (CH_3OH) λ_{max} 278 nm, 382. Anal. ($C_{23}H_{37}N_3OBr_2$·1.5H_2O) C, H, N; Br: calcd, 28.55; found, 29.31.

4-Ethyl-6-methoxy-2-methyl-8-nitroquinoline (10). A mixture of 3 g (0.018 mol) of 4-methoxy-2-nitroaniline (**1**) and 3 g (0.023 mol) of 2-chloropropyl ethyl ketone (**9**)[4] was heated at 80 °C for 50 min in 7 mL of 85% phosphoric acid. Arsenic pentoxide (3 g) was added to the homogeneous mixture and heating with stirring at 85–90 °C continued for 2.5 h. The product was cooled to room temperature, diluted with 50 mL of water, and basified (pH 7.5) with ammonia. The precipitate was collected and thoroughly washed with water and then methanol. The quinoline **10** was obtained as a light orange-brown material: mp 145–147 °C; yield 1.9 g (44%); NMR ($CDCl_3$) δ 1.3 (t, CH_3CH_2), 2.55 (s, CH_3), 2.9 (q, CH_3CH_2), 3.85 (s, OCH_3), 7.03 (s, H_3), 7.25 (d, H_5), 7.37 (d, H_7). Anal. ($C_{13}H_{14}N_2O_3$) C, H, N.

8-Amino-4-ethyl-2-methyl-6-methoxyquinoline (11). Due to the low solubility of **10** in ethanolic HCl, the stannous chloride reduction was carried out in a mixture of equal volumes of THF and ethanol and 3 volumes of hydrochloric acid. The yield was 7.9 g (90%) of **11** from 10 g (0.41 mol) of **10**: NMR ($CDCl_3$) δ 1.25 (t, CH_3CH_2), 2.5 (s, CH_3), 2.82 (q, CH_3CH_2), 3.72 (s, OCH_3), 4.75 (br s, NH_2), 6.35 (s, H_5, H_7), 6.85 (s, H_3).

A portion of this amine was converted to the hydrobromide salt. Recrystallization from a 2-propanol and THF mixture gave the analytical sample, mp 266 °C dec. Anal. ($C_{13}H_{17}BrN_2O$·0.5H_2O).

8-[(4′-Amino-1′-methylbutyl)amino]-4-ethyl-6-methoxy-2-methylquinoline (7c) Diphosphate. Under argon, 3 g (0.014 mol) of **11** was heated at 95 °C bath temperature while a solution of 11 g (0.03 mol) of 4-iodo-1-phthalimidopentane in 3.5 g (0.035 mol) of triethylamine was added over a 6-h period with stirring. The cooled product was extracted with 30 mL of benzene, filtered, and evaporated. The residue was chromatographed on 230 g of silica gel using chloroform as eluent. The yield was 4.1 g of product. This product was refluxed with an excess of hydrazine in ethanol for 1.5 h. After filtering the cooled solution, the ethanol was evaporated and the residue dissolved in methylene chloride. The filtered solution was evaporated in vacuo, leaving **7c** as a yellow syrup (2.2 g, 78%). This was treated with 2.4 g (0.02 mol) of 85% phosphoric acid in ethanol–water. The yellow diphosphate of **7c** (3.6 g, 69%) was collected and recrystallized once from ethanol–water. The salt was obtained as bright yellow crystals, mp 113–115 °C. Anal. ($C_{18}H_{27}N_3O$·2H_3PO_4) C, H, N, P.

8-[[6′-(Diethylamino)hexyl]amino]-4-ethyl-6-methoxy-2-methylquinoline (8c) Dihydrobromide. To 3.4 g (0.016 mol) of **11** heated under argon at 85–90 °C was added slowly a warm solution of 7.5 g (0.024 mol) of 6-(diethylamino)hexyl bromide hydrobromide in 5 mL of DMF over a 4-h period with stirring. Upon cooling to room temperature, the mixture crystallized in part. The product was separated by filtration, washed with ethanol and THF, and air-dried (4.1 g). The filtrate, after evaporation and washing with THF–ethanol, gave some more dihydrobromide (1.6 g). The crude product melted at 210–215 °C, total yield 5.7 g (67%). Two recrystallizations raised the melting point to 220–222 °C dec. Anal. ($C_{23}H_{37}N_3O$·2HBr) C, H, N, Br.

Acknowledgment. The authors express their gratitude to Drs. E. A. Steck and R. E. Strube for many helpful suggestions and discussions during the course of this work. This work was supported by the U.S. Army Medical Research and Development Command under Research Contract DADA-17-74-C-4107. This paper has been designated as Contribution No. 1564 to the Army Research Program on Antiparasitic Drugs.

Role of Iodine in Thyroid Hormones: Molecular Conformation of a Halogen-Free Hormone Analogue

Vivian Cody

Medical Foundation of Buffalo, Inc., Buffalo, New York 14203. *Received November 26, 1979*

The molecular conformation of the halogen-free thyroid hormone analogue, *N*-acetyl-4′-methoxy-3,5,3′-trimethyl-L-thyronine ethyl ester, has been determined by X-ray diffraction techniques. The observed molecular conformation is similar to that found for the natural hormone 3,5,3′-triiodo-L-thyronine (T_3). In this structure, the 3′-methyl group is distal, the overall conformation is cisoid, and the diphenyl ether conformation is twist-skewed. These structural similarities with T_3 show that the conformational features required by the active hormone can still be maintained with methyl substitution. The observation that the halogen-free analogues have relatively high activity but extremely low protein binding affinity implies that the role of iodine in hormone transport and biological activity can be differentiated. These data suggest that the iodines enhance hormone–protein binding by virtue of their electronic, as well as steric, properties.

Many hypotheses of thyroid hormone action have assumed iodine to be an integral part of the requirements for physiological function. This was due in part to the reported inactivity of halogen-free analogues, particularly that of tetramethylthyronine.[1-5] However, recent studies have demonstrated the original synthesis of tetramethylthyronine to be in error.[6] Subsequent testing of

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Table I. Comparison of Thyroid Hormone Activity vs. Protein Binding Affinity

compd	thymocyte[a] act. in vitro, %	antigoiter[b] bioassay in vivo, %	nuclear[b] binding in vitro, %	TBG[c] binding, %
3,5,3′,5′-tetraiodo-L-thyronine (T_4)	30	18	12.5	100
3,5,3′-triiodo-L-thyronine (T_3)	100	100	100	9
3,5,3′,5′-tetramethylthyronine	23	0.54	0.1	0
3,5,3′-trimethylthyronine	23	0.36	0.1	0
3′-isopropyl-3,5-dimethylthyronine	48	3.6	0.7	0.05

[a] Reference 7. [b] Reference 8. [c] Reference 9.

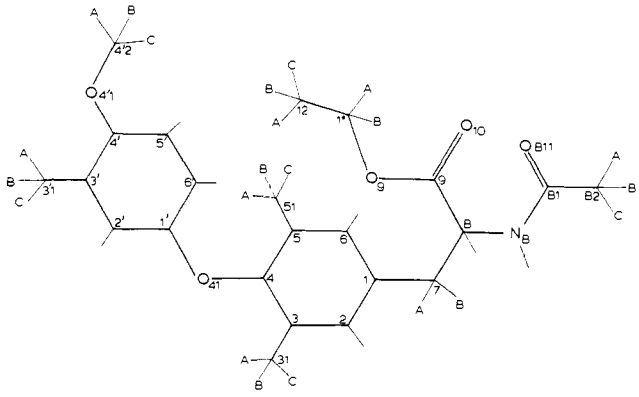

Figure 1. Numbering scheme used for *N*-acetyl-4′-methoxy-3,5,3′-trimethyl-L-thyronine ethyl ester.

the newly synthesized compound shows it to possess significant antigoitrogenic activity, as illustrated in the first column of Table I. These data also show that these halogen-free analogues have limited binding affinity to the nuclear proteins and almost none to the serum proteins. These data further suggest that, while these methyl derivatives are only weakly bound to transport proteins, they do have intrinsic biological activity, implying the need for iodine in hormone transport but not activity. These data now open to question the assumption of a unique functional role of the halogens for thyroid hormone activity.

Another important function of the halogens in enhancing thyroid activity is their ability to achieve and maintain a specific hormone conformation or to direct electronic interactions such as charge–transfer or electron donor effects in molecular associations with binding proteins. One of the primary roles assigned to the inner-ring iodines is that of maintaining a skewed or twist–skewed diphenyl ether conformation about the ether bridge with a C–O–C angle of 120°. This constraint also gives rise to the conformational distinction between distal and proximal 3′ substituents in T_3 analogues.[10,11] Thus, to compare the relative influence of iodine and methyl substituents on thyronine conformation, charge-transfer effects, and intermolecular interactions, a three-dimensional X-ray diffraction analysis of *N*-acetyl-4′-methoxy-3,5,3′-trimethyl-L-thyronine ethyl ester (Figure 1) was undertaken.[12]

Experimental Section

Crystals of the trimethylthyronine derivative were grown at room temperature from ethanol solutions of samples generously supplied by Paul Block, Jr., who synthesized the analogue. Crystal data are listed in Table II. Samples of the unsubstituted parent compound were unstable, and no suitable crystals could be grown.

Table II. Crystal Data for 4′-Methoxy-3,5,3′-trimethyl-L-thyronine *N*-Acetyl Ethyl Ester

molecular formula	$C_{23}H_{29}NO_5$
molecular weight	399.5
crystal system	orthorhombic
space group	$P2_12_12_1$
cell dimensions	
a	9.165(2) Å
b	28.576(3)
c	8.405(2)
Z	4
volume	2200 Å^3
density (calcd)	1.21 g/cm
crystal size	0.16 × 0.16 × 0.48 mm
λ	1.54018 Å
μ	6.96 cm^{-1}
R	3.9% (2320 data)
	4.6% (2606 data)

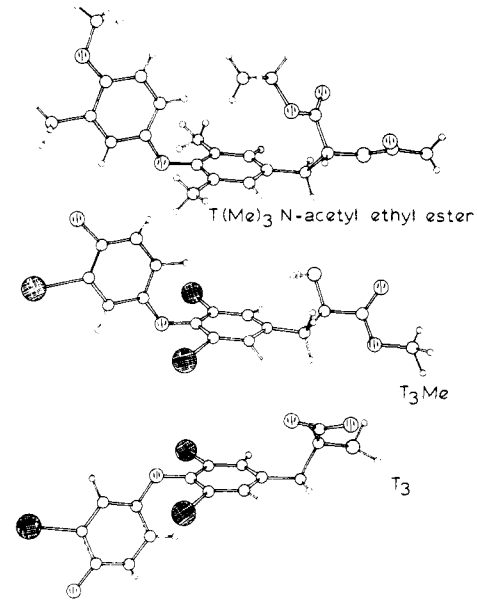

Figure 2. Molecular conformation of the trimethylthyronine derivative and the thyroid hormones T_3 methyl ester and T_3.

Of the 2606 independent reflections, measured in the θ–2θ scan mode on an automatic diffractometer using CuKα radiation at room temperature, 2320 were observed with intensities more than twice their standard deviations. Data were collected on a well-shaped crystal (0.16 × 0.16 × 0.48 mm), which was stable and showed no deterioration upon radiation. The structure was solved by application of MULTAN[13] and NQEST[14] procedures and was

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Table III. Molecular Conformations of Thyroactive Compounds

structure	ϕ,[a] deg	ϕ',[b] deg	χ^1,[c] deg	χ^2,[d] deg	ψ,[e] deg	ref
N-acetyl-4′-methoxy-3,5,3′-trimethyl-L-thyronine, Et ester	−101	25	173	121	40	*f*
3,5,3′-triiodo-L-thyronine	116	−21	−164	76	−7	11
3,5,3′-triiodo-L-thyronine, Me ester	−104	32	−52	121	12	15
3,5,3′-triiodo-L-thyronine, HCl·H_2O	90	−11	56	98	8	16
3,5,3′-triiodothyroacetic acid *N*-diethanolamine	92	−1		78		17
3,5,3′-triiodothyropropionic acid, Et ester	−89	−10		46		18
4′-methoxy-3,5,3′-triiodothyropropionic acid, Me ester	103	−21		99		19
3′-isopropyl-3,5-diiodo-L-thyronine, HCl·H_2O	98	−27		101		20

[a] ϕ = C5-C4-O41-C1′. [b] ϕ' = C4-O41-C1′-C6′. [c] χ^1 = C1-C7-C8-N8. [d] χ^2 = C2-C1-C7-C8. [e] ψ = N8-C8-C9-O10. [f] This work.

refined by full-matrix least-squares techniques, using anisotropic thermal parameters for the non-hydrogen atoms. Positional parameters for all hydrogen atoms were located from difference Fourier maps and were refined isotropically. The final residual $R = \sum||F_o| - |F_d||/\sum|F_o|$ was 0.039 for the observed data. Positional and anisotropic thermal parameters for all non-hydrogen atoms, positional and isotropic parameters for hydrogen atoms, calculated bond distances and angles, lists of calculated structure factors, and detailed packing diagrams are available (see paragraph at end of paper regarding supplementary material).

Results

The molecular conformations of the trimethylthyronine derivative and two thyroid hormones are illustrated in Figure 2. As also shown in Table III, the molecular conformation is similar to that observed for the parent hormone T_3 and its derivatives. The 3′-methyl group is in the distal conformation as previously observed in the crystal structures of T_3[11] and its methyl ester.[15] The diphenyl ether conformation, defined by the torsional parameters ϕ and ϕ', is twist-skewed[17] as observed for the thyronine structures. This is in contrast to a skewed ($\phi,\phi' = 90,0°$) conformation adopted by the thyroactive acids.

The positioning of the outer phenyl ring and the alanine group, on the same side of the inner phenyl ring (Figure 2), defines the cisoid conformation, which is characterized by a negative value of ϕ (Table III). The alanine group is nearly perpendicular to the inner phenyl ring (χ^2), a characteristic of aromatic amino acids,[21] and the amine function is fully extended (χ^1) with respect to the ring system. The ethyl ester has an extended conformation (C9-O9-C11-C12 = 180°) and is folded back over the inner ring. The 4′-methoxy group is coplanar with the outer phenyl ring and is trans to the 3′-methyl, as observed in similar iodinated thyroid analogue structures.[19,22] There is only one hydrogen bond in the structure, N-H··O, from the amine to the carbonyl oxygen of the *N*-acetyl of an adjacent molecule (N··O = 2.94 Å).

Discussion

Two roles ascribed to iodine in the enhancement of hormone activity are the ability to maintain a specific conformation and to participate in electronic interactions. An example of the constraints placed on the diphenyl ether conformation by the presence of the inner ring iodines is shown in Figure 3. The structure of thyronine ethyl ester,[23] which has only 3,5-hydrogens, is observed with a twist diphenyl ether conformation, whereas that of 3,5-diiodothyronine[24] is twist-skewed as found for the hormones T_4 and T_3. The observation of a twist-skewed, distal 3′-methyl conformation for this trimethylthyronine analogue indicates that methyl substituents have sufficient bulk to maintain this perpendicular arrangement of the phenyl rings. Also, as illustrated in Figure 4, the diphenyl ether conformation of the trimethyl analogue is almost indistinguishable from that of the hormone T_3 as its methyl ester. These data reemphasize the steric specificity of the thyromimetic compound for activity.

The observation that the halogen-free analogues (Table I) show significant hormonal activity but have weak protein affinities suggests that the unique electronic characteristics of the halogens are more important to hormone transport than to physiological function. These data also indicate that, while methyl substitution can provide the required

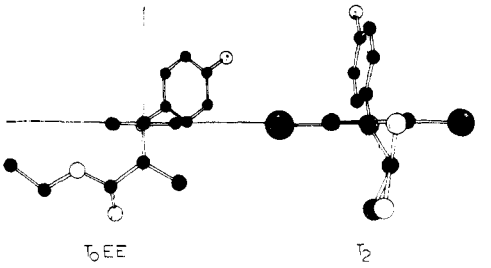

Figure 3. Comparison of the conformation of thyronine ethyl ester with that of diiodothyronine. This view is a projection down the vector from C1 to C4 of the inner phenyl ring.

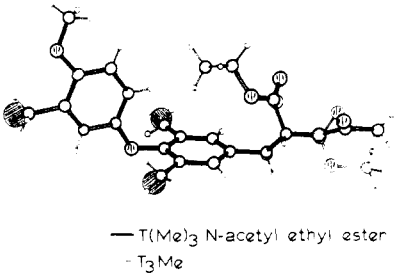

Figure 4. Superposition of trimethylthyronine derivative (dark) over that of the methyl ester of T_3.

(15) V. Cody, *J. Med. Chem.*, 18, 126 (1975).
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conformation for hormonal activity, it does not have the electronic characteristics which make halogenated structures better protein binders. On the basis of these binding and activity data,[2,3] the concept of the hormone molecule acting as a structurally specific matrix was developed as opposed to a particular functional portion of the molecule being involved in hormone action. Thus, the unique contribution of iodine to the thyroid hormone may be in biosynthesis and metabolism where evolution favored a system with ready access to iodine.[3]

In conclusion, this crystallographic determination of the first halogen-free thyroactive analogue shows the molecular conformation to be the same as the active natural hormone, T_3. These data show that methyl substituents are sufficiently large to maintain the conformational constraints required by the active hormone.

Acknowledgment. Supported in part by Grant AM-15051 awarded by the National Institute of Arthritis, Metabolism and Digestive Diseases, DHEW. The author thanks Q. Bright, E. DeJarnette, and J. Hazel for technical assistance.

Supplementary Material Available: Positional and anisotropic thermal parameters, bond distances and angles, calculated structure factors, and packing diagram (16 pages). Ordering information is given on any current masthead page.

Book Reviews

Metal Ions in Biological Systems. Volume 8. Nucleotides and Derivatives: Their Ligating Ambivalency. Volume 9. Amino Acids and Derivatives. Edited by Helmut Sigel. Marcel Dekker, New York. 1979. Volume 8: xix + 232 pp. 16 × 23 cm. $29.75. Volume 9: xix + 277 pp. 15 × 23.5 cm. $34.50.

Volume 8, dealing with the complexation of nucleosides and nucleotides with metal ions, will be of considerable interest to scientists concerned with nucleic acid biochemistry and gene-related biological effects of metals. It will provide basic background for two future volumes in this series dealing specifically with metal carcinogenesis and anticarcinogenesis.

The first chapter reviews the X-ray structural studies of metal complexes of nucleoside, polynucleotide, and nucleoside–peptide mixed complexes. It is very well illustrated and gives a good summary with some useful generalizations about preferred modes of coordination for various metals and nucleoside derivatives *in the solid state*. Chapter 2 extends this to the solution phase. It is a highly recommendable discussion of proton binding sites and proton basicity vs. the metal coordination behavior of bases, nucleosides, and nucleotides. Kinetic, pH, and hard/soft effects are brought out and illustrated well, and a concise discussion of the power and limitations of various methods for studying metal ion–nucleoside derivative interactions in solution is given. In a similar vein, Chapter 3 covers metal binding by base-modified nucleosides, particularly *N*-oxides and several nucleoside/base "antimetabolites". Chapter 4 deals with the application of principles developed in the first three chapters to the problem of developing base-specific heavy-metal "stains" for visualization and sequencing of polynucleotides and nucleic acids by electron microscopy. It is a very interesting, if somewhat specialized, discussion. The last chapter dealing with "macromolecules of biological interest in complex formation" appears a bit out of place in this volume, as it does not deal with nucleic acids. Its main point concerns cooperativity effects in multimetal binding and the formation of binding sites involving groups close in *space* but not in *sequence* in the macromolecules. Included in the latter are the prothrombin/Ca^{2+} system, albumin, insulin, and some synthetic polymers.

Overall this volume conforms to the standard set by earlier volumes in this useful series. While the last three chapters will probably be of greatest interest to more specialized readers, the first two chapters may be recommended for a very broad readership among scientists whose interests include metal ions in biological systems, in general, or nucleic acid chemistry and biochemistry, in particular.

The theme of Volume 9 closely parallels that of the previous volume on nucleosides and derivatives, and the tone is clearly set by the opening chapter dealing with complexation of metal ions by amino acids with chelatable side-chain donor groups. Eighteen such amino acids are identified and twelve of these are given a case-by-case review, being treated as substituted glycines. The much overworked series Mn–Zn is again the principal focus, but other metals receive some mention. The remaining six "glycine derivatives" are covered in the next four chapters in terms of X-ray structural studies and solution studies involving equilibrium and spectroscopic methods. Included in this group are aspartate and glutamate (Chapter 2), L-cysteine and D-penicillamine (Chapter 3), glutathione (Chapter 4), and L-Dopa (Chapter 5). In the last three chapters, the additional complication of ligand redox chemistry and its metal catalysis or inhibition is also dealt with, a much broader range of metals is considered, and quite a bit of tabulated data is presented. Chapter 6 covers complexation of metals by two or more polydentate ligands, including a discussion of factors such as ring size, strain, and flexibility, enthalpic vs. entropic effects, and stereoselectivity and methods for its detection. The final chapter deals with specific studies of metal complexation by corticotropin (ACTH) and basic bovine pancreatic trypsin inhibitor, two medium-sized peptides with well-defined structures.

Each of the chapters is well written and quite readable. The chapter on L-Dopa will be useful to medicinal chemists and pharmacologists interested in catecholamines and those on cysteine and glutathione will interest toxicologists concerned with metal compounds, but, in general, this volume is more for the specialist or the reference collection than for those looking for a more didactic approach to this subject as found in earlier volumes in this series.

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Membrane Proteins in Energy Transduction. Edited by R. A. Capaldi. Marcel Dekker, New York. 1979. xi + 526 pp. 16 × 23 cm. $48.50.

This book summarizes the extent of current research mainly on mitochondrial proteins, with chapters on photosynthetic reaction centers (J. M. Olson and J. P. Thornber), energy transducive components of *E. coli* (P. D. Bragg), and the purple membrane of *H. halobium* (J. K. Lanyi). Titles of other chapters are "Mitochondrial Iron–Sulfur Flavodehydrogenases" (T. Ohnishi), "Succinate–Cytochrome *c* Reductase Complex of the Mitochondrial Electron Transport Chain" (B. L. Trumpower and A. G. Katki), "Structure of Cytochrome *c* Oxidases" (R. A. Capaldi), and "The Mitochondrial ATPase" (A. E. Senior).

This book is supposed to provide an up-to-date compilation of investigations in specialized research area by experts. Like many edited volumes, this book lacks a certain degree of organization and continuity, since the last three chapters have little connection and/or relevance to those that precede them. However, the individual coontributions are generally very good and contain references through 1976 or 1977.