tion. Conventional clearance formulas were used, and solutes in urine and plasma were measured (*10*). The clearances of creatinine and inulin were identical when compared in affected dogs.
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Molecular Conformation of a Halogen-Free Thyroxine Analog: 4′-Methoxy-3,5,3′-trimethyl-L-thyronine *N*-Acetyl Ethyl Ester

Abstract. *The molecular conformation of the halogen-free thyroxine analog 4′-methoxy-3,5,3′-trimethyl-L-thyronine* N-*acetyl ethyl ester has been determined by x-ray diffraction techniques. The unsubstituted parent compound, trimethylthyronine, has significant biological activity in rat thymocyte tests when compared with the thyroid hormone 3,5,3′-triiodo-L-thyronine (T_3). Although no activity data are available for the analog studied, it is presumed to be inactive because of the 4′-methoxy blocking group. The observed conformation of this structure is similar to that found for the natural hormone T_3. The 3′-methyl group is distal, the overall conformation is cisoid, and the diphenyl ether conformation is twist-skewed. The results of this diffraction study show that methyl substituents are capable of maintaining the thyronine conformation required for hormonal activity; they suggest that iodine enhances hormone-protein binding because of the electronic effects it produces either by alteration of molecular charge distributions or by direct charge-transfer interactions with the serum or nuclear binding proteins.*

Since the thyroid hormones thyroxine (T_4) and 3,5,3′-triiodo-L-thyronine (T_3) are the only naturally occurring iodinated compounds known to have vital biochemical activity, it is presumed that iodine plays an essential role in hormone activity. Some investigators have proposed that enhanced thyroid activity may be related to the ability of iodine to (i) achieve and maintain a specific hormone conformation or (ii) direct electronic interactions such as charge-transfer or electron donor effects in molecular associations. The observed antigoitrogenic activity (*1*–*7*) of such nonhalogenated thyroxine analogs as 3,5,3′,5′-tetramethylthyronine (Me_4) and 3,5,3′-trimethylthyronine (Me_3) implies a distinction between these proposed functional roles of iodine and throws light on their significance for hormonal activity.

The results of studies measuring thyromimetic activity and protein binding affinities of these methylthyronines suggest that the role of iodine in functional activity and protein binding can be differentiated. In rat thymocyte activity tests (*5*) the methylthyronines had 23 percent of the activity of T_3, and in tadpole assays (*4*) they had 15 percent of the activity of T_4, but in rat antigoiter tests Me_3 had only 2 to 3 percent of the activity of T_4 (*4*). In studies of binding affinities for the serum protein thyroxine binding globulin, the methylthyronines were even weaker (*6*). The relatively high activity in the thymocyte tests and the extremely low serum protein binding affinities suggest that these methyl derivatives cannot be transported, but do have intrinsic biological activity, implying the need for iodine in hormone transport but not activity.

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 4′-methoxy-3,5,3′-trimethyl-L-thyronine *N*-acetyl ethyl ester (Fig. 1) was undertaken.

Samples of the trimethylthyronine derivative (*8*) were crystallized from ethanol solutions at room temperature. The crystals are orthorhombic, space group $P2_12_12_1$, with unit cell parameters a = 9.165(2), b = 28.576(3), and c = 8.405(2) Å, Z = 4 molecules, and volume = 2200 Å^3. Samples of the unsubstituted parent compound were unstable and no suitable crystals could be grown.

Of the 2606 independent reflections, measured in the θ-2θ scan mode on an automatic diffractometer using Cu Kα radiation, 2320 were observed with intensities more than twice the standard deviations. Data were collected on a well-shaped crystal (0.16 × 0.16 × 0.48 mm), which was stable and showed no deterioration on radiation. The structure was determined by application of the MULTAN (*9*) and NQEST (*10*) procedures and was refined by full-matrix least-squares techniques, using anisotropic thermal parameters for the nonhydrogen atoms to a current residual of R = 4.1 percent.

The molecular conformation of the trimethylthyronine derivative is shown in Fig. 2 and the conformational parame-

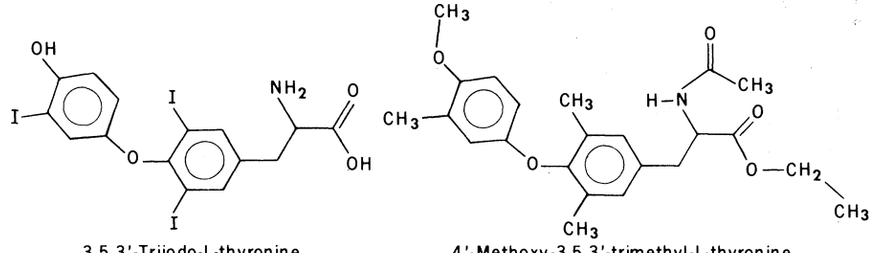

Fig. 1. Comparison of T_3 with the trimethylthyronine analog.

Table 1. Conformation of thyroactive compounds.

Structure	3′ Conformation	Overall conformation	ϕ*	ϕ'†	Reference
4′-Methoxy-3,5,3′-trimethyl-L-thyronine *N*-acetyl ethyl ester	Distal	Cisoid	−103°	25°	
3,5,3′-Triiodo-L-thyronine (T_3)	Distal	Transoid	116°	−21°	(*12*)
3,5,3′-Triiodo-L-thyronine methyl ester (T_3Me)	Distal	Cisoid	−104°	32°	(*13*)
3,5,3′-Triiodothyroacetic acid	Distal	Transoid	92°	− 1°	(*18*)
3,5,3′-Triiodo-L-thyronine H_2O HCl	Proximal	Transoid	90°	−11°	(*19*)
3′-Isopropyl-3,5-diiodo-L-thyronine HCl H_2O	Proximal	Transoid	90°	−27°	(*20*)
3,5,3′-Triiodothyropropionic acid ethyl ester	Proximal	Cisoid	− 89°	−10°	(*21*)
4′-Methoxy-3,5,3′-triiodothyropropionic acid methyl ester	Proximal	Cisoid	−101°	21°	(*22*)

*ϕ = C5-C4-O4-C1′. †ϕ' = C4-O4-C1′-C6′.

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ters are listed in Table 1 (*11*). The overall conformation is similar to that observed for the parent hormone T_3 and its derivatives.

The 3′-methyl group is distal to the inner ring—that is, pointed away from the inner ring—as observed in the structures of the natural hormone T_3 (*12*) and its methyl ester (*13*) (Fig. 3). Among the thyronine analogs whose x-ray crystal structures have been determined, four have been observed to have the distal and four the proximal (pointed toward the inner ring) 3′ substituent (*14*). Energy calculations for T_3 (*15*) suggest that the rotational energy barrier between the distal and proximal conformations is small (11 kcal), and nuclear magnetic resonance studies (*16*) suggest that the distal/proximal ratio in solution is 0.56. Measurement of the thyromimetic activity and relative binding affinities of several thyroactive analogs has shown that it is the distal conformer that is hormonally active (*6*, *17*). The observation of a distal 3′-methyl group in this structure suggests that methyl substitution on the inner phenyl ring is capable of stabilizing the active distal conformer.

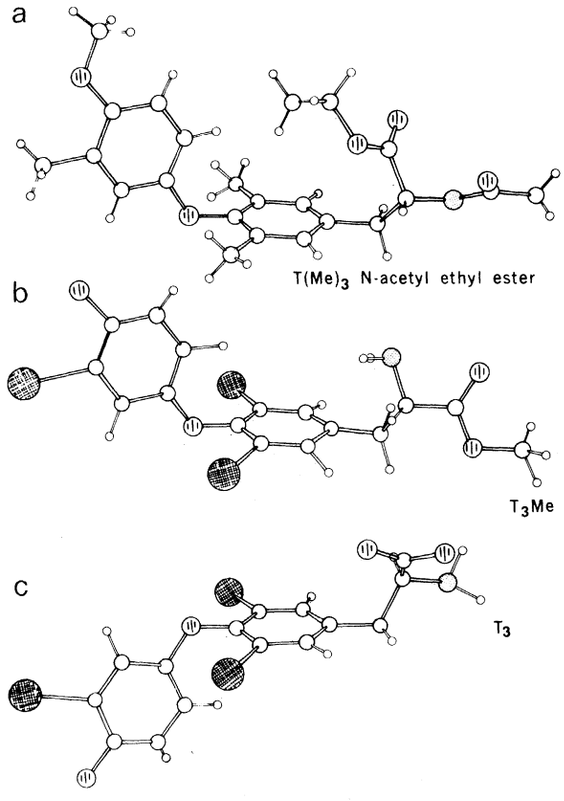

Fig. 2. Molecular conformation of (a) 4′-methoxy-3,5,3′-trimethyl-L-thyronine *N*-acetyl ethyl ester, (b) 3,5,3′-triiodo-L-thyronine methyl ester, and (c) 3,5,3′-triiodo-L-thyronine.

The diphenyl ether conformation is twist-skewed (distorted from perpendicular ring system), as indicated by the parameters ϕ and ϕ', which are near the average of observed values in thyronine structures ($\phi/\phi' = 108°/28°$), while thyroactive acid compounds (NH_2 group removed) are observed in a skewed ($\phi/\phi' = 90°/0°$) (perpendicular ring system) conformation (*18*) (Fig. 4). One of the primary roles assigned to the inner ring iodines was that of maintaining a skewed or twist-skewed diphenyl ether conformation. The observation of a twist-skewed conformation in this structure (Table 1) indicates that methyl substituents have sufficient bulk to maintain this conformation.

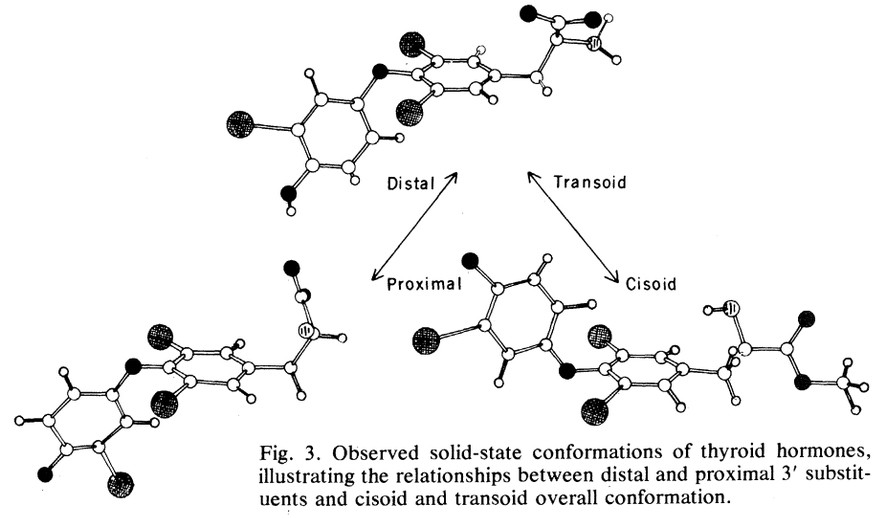

Fig. 3. Observed solid-state conformations of thyroid hormones, illustrating the relationships between distal and proximal 3′ substituents and cisoid and transoid overall conformation.

The positioning of the outer phenyl ring and the alanine group, on the same side of the inner phenyl ring (Fig. 3), defines the cisoid conformation, which is characterized by a negative value of ϕ (Table 1). The alanine group is nearly perpendicular to the inner phenyl ring, and the amine function is in a fully extended position with respect to the ring system. The methoxy group on the 4′-OH is coplanar with the ring and *trans* to the 3′-methyl group. There is only one hydrogen bond in the structure, N-H···O, from the amine to the carbonyl oxygen of the N-acetyl group of an adjacent molecule (N···O = 2.94 Å).

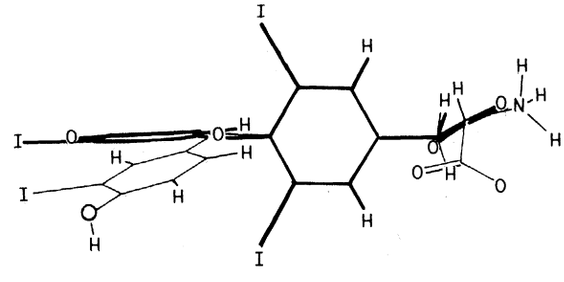

Fig. 4. Superposition of a skewed (dark) diphenyl ether conformation and a twist-skewed (light) conformation.

To my knowledge, this is the first structural observation of a halogen-free thyroxine analog. The observation of a distal 3′-methyl and a twist-skewed diphenyl ether conformation in this structure shows that methyl substituents are sufficiently large to maintain the biologically active form of the molecule and that iodine is not essential to these functions. Consequently, the iodines may enhance hormone-protein binding by more

direct interaction such as charge-transfer or redistribution of electronic charge within the thyronine molecule.

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Rod-Cone Dysplasia in Irish Setters: A Defect in Cyclic GMP Metabolism in Visual Cells

Abstract. *An abnormality in retinal guanosine 3',5'-monophosphate (cyclic GMP) metabolism is demonstrated in the inherited rod-cone dysplasia of Irish Setter dogs. Affected visual cells are deficient in cyclic GMP phosphodiesterase activity and have elevated levels of cyclic GMP. The biochemical abnormalities observed in affected retinas of Irish Setters are similar to those in the retinas of mice with inherited retinal degeneration before visual cell degeneration begins. A defect in cyclic GMP metabolism may be characteristic of early-onset degenerative diseases of the retina, possibly including those that affect humans.*

Retinal degenerations that cause blindness are known to occur in several species of animals, including humans (*1*). In certain of these diseases, photoreceptor cells of the retina degenerate selectively before reaching maturity. These early-onset disorders (dysplasias) are inherited, and some may share a common biochemical etiology.

Detailed studies on mice with inherited retinal degeneration (*rd* mice) have shown that an abnormality in the metabolism of guanosine 3',5'-monophosphate (cyclic GMP) occurs before visual cells begin to degenerate (*2*). The metabolic abnormality is related to a deficiency in cyclic GMP–phosphodiesterase activity that results in the accumulation of cyclic GMP within affected photoreceptor cells (*3*). A causal relationship between the elevation of cyclic GMP and photoreceptor cell degeneration has been confirmed in vitro in normal eye rudiments of *Xenopus laevis* cultured in the presence of phosphodiesterase inhibitors (*4*).

Now we report that a derangement in cyclic GMP metabolism is present in the dystrophic retina of Irish Setters, and that the defect is biochemically similar to that of *rd* mice. This raises the possibility that a defect in cyclic GMP metabolism can exist in retinal degenerative diseases in other species including man.

Irish Setters carry an autosomal recessive mutation (*5*) that causes retinal degeneration (rod-cone dysplasia) in homozygous animals (*6*). Electroretinographic measurements have proved useful for establishing the degree of rod or cone degeneration in living animals and, therefore, the progress of the disease. In vivo, the physiological response of affected rods to light is greatly diminished during early postnatal life, while that of cones is altered to a lesser extent. Morphologic studies have demonstrated failure of postnatal differentiation of visual cells in affected retinas. Following their initial development, affected visual cells soon appear pathological and form only small and disorganized outer segments. By 18 to 20 weeks, all rod cells have degenerated, leaving the retina with only a small number of cone cells in the photoreceptor layer.

Affected Irish Setters (8 and 12 weeks of age) were studied physiologically to assess visual cell function, morphologically to determine visual cell pathology, and biochemically to assess whether cyclic nucleotide metabolism was normal. Unaffected heterozygote Irish Setters of similar age were used as control animals. These animals were considered the best available control subjects; their morphology and retinal cyclic GMP content were comparable to those of two other normal dog breeds (beagles and foxhounds). Electroretinograms were recorded from halothane-anesthetized animals; in affected dogs, rod-mediated responses to light were completely absent. Cone responses were present but abnormal; the response amplitude was reduced and the peak latencies were increased. All responses were normal in the control animals.

Morphologic studies revealed that the visual cells from the retinas of affected dogs were abnormal at both 8 and 12 weeks of age (Fig. 1). The photoreceptor layer contained a minimum of rod outer segment material, short inner segments, and fewer visual cells than that of a control retina. Cones were prominent, their inner segments broad and club-shaped, the outer segments short and abnormal. The inner retinal layers remained unchanged and normal in appearance. In the Irish Setter disease, the early onset of rod degeneration and the maintenance of cone integrity is thus similar to the pattern of visual cell degeneration observed in retinas of *rd* mice and comparable to that suggested from electrophysiological studies of the human disease, retinitis pigmentosa (*7*).

Cyclic nucleotide metabolism in control dog retinas appears to be similar to that of other species (Table 1) in that retinal homogenates show two apparent values of the Michaelis constant (K_m) for phosphodiesterase with cyclic GMP as substrate. The phosphodiesterase with the high value (K_m-A) has been localized within the photoreceptor layer of the retina of several animals, and that with the lower value (K_m-B) is associated with the inner layers (*8*). In affected Irish Setters, the activity of K_m-A is below the level of detection, and that of the inner retina is comparable to the control. Therefore, a deficiency in cyclic GMP-phosphodiesterase activity can be demonstrated in the affected retinas at the time of photoreceptor cell degeneration.

The deficiency in cyclic GMP–phosphodiesterase activity of affected visual cells impairs their ability to hydrolyze cyclic GMP. The concentration of cyclic GMP in the retinas of affected dogs is about ten times greater than that in control retinas (Table 1). In affected retinas, however, the deficiency in phosphodiesterase activity does not alter the concentration of adenosine 3',5'-mono-

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