concentration, four subjects were given a potent diuretic (furosemide, 40 mg orally). During these studies the serum osmolality remained within 2 percent of the control value, and the serum prolactin concentration remained stable.

In man, changes in serum osmolality were accompanied by parallel changes in serum prolactin concentration. Administration of ovine prolactin to humans has been shown to result in a marked antidiuresis (*9*). Hypertonic saline infusion has been reported to cause a massive increase in serum prolactin in a single patient with a pituitary tumor and galactorrhea (*13*). We observed that hypertonic saline administration to normal subjects results in an increase in serum prolactin concentration which may facilitate the appropriate renal retention of water. Conversely, hypotonic saline administration resulting in suppression of endogenous prolactin secretion may facilitate the appropriate diuresis that occurs. The full significance of these observations in clinical medicine, especially the role of prolactin in volume-depleted and volume-expanded states, will require further investigation.

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Distal Conformation of the Thyroid Hormone 3,5,3′-Triiodo-L-Thyronine

Abstract. *In the crystal structure of the thyroid hormone, 3,5,3′-triiodo-L-thyronine, the 3′ iodine is observed in the* distal *position, away from the alanine-bearing ring of the thyroid hormone. This result had been anticipated from stereochemical and biological activity studies. However, previous observations of structures in which the 3′ iodine was* proximal *had cast some doubt on the stability of the 3′* distal *conformation. This observation suggests that the relative energies of the two conformers is similar and that perhaps the barrier to rotation is not as great as previously supposed since both the* distal *and* proximal *conformers have now been observed in the solid state.*

In the study of structural requirements for maximal thyromimetic activity, one of the most pertinent questions concerns the preferred conformation of the hormone triiodothyronine (T_3). Because the chemically identical 3′ and 5′ positions of the outer (β) ring of T_3 are not conformationally equivalent, as shown by Jorgensen and his co-workers (*1*), it is uncertain whether the preferred conformation of T_3 is with the 3′ iodine in the *distal* or *proximal* conformation. To verify the importance of this conformational feature, numerous structural analogs of thyroxine were synthesized (*2*) in an effort to determine the structural features that cause the stimulation or suppression of various physiological functions by thyroid hormones. From these stereochemical and biological activity studies, it appears that hormonal activity is greater for the *distal* orientation of the 3′ iodine. The requirement of a *distal* 3′ substituent for activity was further substantiated in a study of the binding of thyroxine-binding globulin to the thyroxine analogs 3,5-diiodo-2′,3′-dimethylthyronine and 3,5-diiodo-2′,5′-dimethylthyronine as test examples where either the *distal* or *proximal* conformation is locked by the steric hindrance of the 2′ substituents (*3*). This investigation showed that the *distal* analog had an almost twofold greater binding affinity for thyroxine binding globulin than the *proximal* form.

The studies of Kier and Hoyland (*4*) on molecular orbital energy calculations of trisubstituted thyronines suggest a perpendicular arrangement of the phenyl rings "locked in" by a considerable barrier to internal rotation. Their calculations do not show any significant preference for the *distal* or *proximal* form. Their results are not in agreement with the molecular orbital calculations made by Camerman and Camerman (*5*), who found that the total energy for the *proximal* T_3 is lower than that computed for the *distal* T_3. Earlier crystallographic verification of the mutually perpendicular arrangement of the two phenyl rings about the 120° ether linkage was first observed in the structure of diiodothyronine (*6*) and again in the structure of triiodothyronine HCl (*5*).

We report here a crystallographic observation of a triiodothyronine in which the 3′ iodine is in the *distal* conformation, as anticipated from stereochemical studies (*7*). The observation of the *distal* conformation in the crystal structure of 3,5,3′-triiodo-L-thyronine as well as in the crystal structures of 3,5,3′-triiodothyroacetic acid *N*-diethanolamine (1:1) complex (*7*) and 3,5,3′-triiodo-L-thyronine methyl ester (*8*) and the observation of the *proximal* conformation in the crystal structures of 3,5,3′-triiodothyronine HCl (*5*) and 3,5,3′-triiodothyropropionic acid ethyl ester (*9*) indicates that both forms are readily accessible in solution.

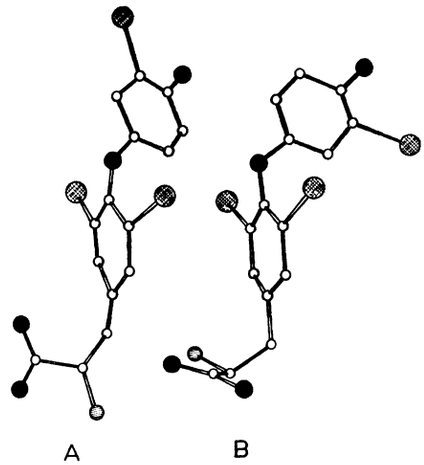

Fig. 1. Diagrams of triiodo-L-thyronine illustrating (A) the *distal* and (B) the *proximal* conformation.

Crystals of 3,5,3′-triiodo-L-thyronine were grown at room temperature from an ethanol solution containing an excess of salicylic acid. The crystal system is monoclinic $P2_1$, $Z=2$, with dimensions $a=13.891_4$ Å, $b=5.999_1$ Å,

$c = 12.264_3$ Å, and $\beta = 116.81_4°$. Intensities for 1725 (1558 observed) reflections with $2\theta < 50°$ were collected (General Electric XRD-5 diffractometer; MoKα radiation monochromatized by balanced zirconium and yttrium filters).

The iodine atoms were located from Patterson functions, and the complete molecule from three-dimensional Fourier synthesis. After block diagonal least-squares refinement of all observed data, the reliability index was 0.049.

The observed conformation of the *distal* triiodo-L-thyronine is shown in Fig. 1 along with that of the *proximal* form of triiodo-L-thyronine observed by Camerman and Camerman (*5*). Again the two phenyl rings are nearly perpendicular to each other, and the dihedral angles between the α ring and the C–O–C ether plane and the β ring and the C–O–C ether plane are 116° and 20°, respectively. The C–O–C angle is 120°. The structure is hydrogen bonded in the lattice by a network of hydrogen bonds through the carboxyl group and the amine of adjacent molecules. There are no short intermolecular distances involving the 3′ iodine in T_3 nor in the other two structures having the 3′ iodine in the *distal* position.

The observation of both the *distal* and *proximal* forms of T_3 in the solid state indicates that the two conformations are readily accessible in solution and that their relative energies are probably similar. This assumption has been further verified by Kollman (*10*) who, from molecular orbital calculations, found that the *proximal-distal* energy difference was on the order of 0.1 kcal/mole. The three crystalline examples of the *distal* conformation in various compositional derivatives of T_3 as opposed to the two *proximal* forms of T_3 crystallized from HCl points to the ease with which these two conformers can be selectively crystallized and that solvent effects may have some influence in stabilizing either form.

Because the *distal* conformation of T_3 has been shown to be stable, it is not necessary to propose that the rotation of the β ring is part of the T_3 receptor function (*5*). Furthermore, the readily accessible *distal* conformation does not throw into question the results of biological activity studies, nor does it require a modification for the manner of tissue uptake of T_3.

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12,13-Epoxy-Δ^9-Trichothecenes as the Probable Mycotoxins Responsible for Stachybotryotoxicosis

Abstract. Stachybotrys atra *cultures grown on oats produced five compounds toxic to brine shrimp; three are the sesquiterpenoid mycotoxins known as 12,13-epoxy-Δ^9-trichothecenes. One trichothecene is roridin E, a known metabolite of* Myrothecium verrucaria. *The other two were hydrolyzed to verrucarol, the product of roridin and verrucarin hydrolysis. Spectroscopic data indicate that the two remaining compounds are also 12,13-epoxy-Δ^9-trichothecenes. These metabolites are probably among those responsible for stachybotryotoxicosis.*

Stachybotryotoxicosis is a mycotoxicosis caused in animals by the ingestion of feed contaminated with certain toxin-producing strains of *Stachybotrys atra* (synonym *S. alternans*). The first reports of the disease came from the U.S.S.R. in 1931; the toxicosis attained enzootic proportions in some areas of the U.S.S.R. and was responsible for the deaths of thousands of horses. After an intensive search the cause of the toxicosis was attributed to certain metabolites of *S. atra*; these compounds have heretofore resisted chemical characterization (*1*).

More recently, Palyusik (*2*) has reported outbreaks of stachybotryotoxicosis among poultry, horses, and swine in Hungary. Forgacs and co-workers (*3*) have shown that the toxicosis can affect calves, sheep, swine, dogs, guinea pigs, rabbits, and mice.

Investigations of the mycotoxicosis have been reported and reviewed by Forgacs (*1*) and Palyusik (*2, 4*). Forgacs describes two forms of the toxicosis in horses: the typical, which is divided into three stages, and the atypical or shock form with one stage. In the typical form, the first stage involves stomatitis followed by fissures at the corner of the mouth, progressing to necroses. These lesions may subside before onset of the second stage, which is characterized by thrombocytopenia and increased blood clotting time. In some cases the blood fails to clot. In the third stage, thrombocytopenia and leukopenia increase, and failure of the blood to clot is observed in all cases. Body temperature increases, fresh necrotic areas appear, frequently with bacterial infection, and the animal usually dies within 1 to 6 days.

The atypical or shock form develops after ingestion of larger quantities of the toxin. Forgacs and co-workers (*3*) induced this form in one horse after it consumed 0.5 lb (1 kg = 2.2 lb) of straw infected with *S. atra* over a period of 10 days. Nervous disorders are the major visible symptoms of this form, which generally terminates in death.

Some of the symptoms produced in experimental animals as reported by Forgacs (*1*) and Palyusik (*2, 4*) are similar to those reported for the 12,13-epoxy-Δ^9-trichothecene group of toxic fungal metabolites; similar symptoms are dermal necrosis after topical application, leukopenia, thrombocytopenia, hemorrhages in the entire length of the digestive tract, and a noticeable lag period between the time of dosing and the appearance of visible symptoms. These symptoms varied with the test animals, quantities used in dosing, method of dosing, and observations made by the investigators. These observations led Bamburg and Strong (*5*) to postulate that the condition was caused by some members of the 12,13-epoxy-Δ^9-trichothecenes, a group of closely related sesquiterpenoids produced by various species of imperfect fungi.