Thyroxine Analogues*

VII. ANTIGOITROGENIC, CALORIGENIC, AND HYPOCHOLESTEREMIC ACTIVITIES OF SOME ALIPHATIC, ALICYCLIC, AND AROMATIC ETHERS OF 3,5-DIIODOTYROSINE IN THE RAT†

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In an effort to establish minimal and optimal structural requirements for the stimulation or suppression of various physiological functions by the thyroid hormones, and to gain knowledge of the structural and functional nature of the hormone receptor(s), a study has been made of the effects on various biological responses during a systematic alteration of the outer ring of thyroxine (Fig. 1). The side chain and inner ring of thyroxine have been varied widely in previous studies, with maximal activity in mammals being shown by analogues possessing the 3,5-diiodotyrosinyl residue (ether oxygen, inner ring, side chain) (2, 3). This portion of the molecule has therefore been held constant in the compounds prepared (4–6) for the present study of outer ring requirements.

It has been suggested that the side chain of thyroxine and its analogues may be associated with transport and binding to the hormone receptor, with the outer ring being involved in subsequent events leading to the hormonal response (7–9). The hypothesis of Niemann (10), involving reversible electron transport, illustrates one way in which this might occur. It has been our working hypothesis that the entire 3,5-diiodotyrosinyl moiety is associated with transport and receptor binding, with the outer ring and ether oxygen comprising the functional unit in initiating the various hormonal responses.

The assay methods used represent a variety of physiological responses elicited by the thyroid hormones: (a) oxygen consumption, a probable measure of the increase in production of energy by most cells; (b) goiter prevention, a measure of the inhibition of thyrotropin release from the pituitary; and (c) blood cholesterol levels, a measure of the effect on cholesterol degradation and excretion. Previous studies (11, 12) have shown partial dissociation of such effects.

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It has been generally accepted that the diphenyl ether (or thio ether) nucleus is indispensable for thyroxine-like activity. The principal basis for the assignment of this structural requirement has been the inactivity of compounds in which the ether bridge was absent or extended by methylene groups (2, 3). Compounds without the diphenyl ether structure, such as alkyl and aralkyl ethers of N-acetyl-3,5-diiodo-L-tyrosine and related compounds, have been reported to be active as thyroxine antagonists in both tadpole (7, 13) and mammalian (14–17) assays.

To extend these observations by testing the effect on thyrinous and antagonistic activity in mammals of analogues possessing nonaromatic substituents in place of the outer ring, a series of aliphatic and alicyclic ethers of 3,5-diiodotyrosine was prepared (6). The aliphatic series included relatively nonpolar groups (n-butyl, allyl) as well as those capable of forming ionic and hydrogen bonds (—COOH, —N(CH₃)₂) and spaced at varying distances from the diiodophenyl ether nucleus by connecting methylene chains. It was hoped that these groups might provide a more firmly bound drug-receptor complex and act as antagonists, if they did not carry the functional ability to act like thyroxine. Alicyclic derivatives, including the cyclohexyl, 3-cyclohexenyl, and 6-hydroxy-3-cyclohexenyl ethers, were prepared to test the functioning of a large, fairly planar bulk of varying polarity in place of the outer ring.

Following the conclusion (see “Results and Discussion”) that aromatic character is a probable requisite for thyroxine-like activity for the responses tested in the rat, the nature of steric factors related to substituents on the outer phenyl ring were further investigated. It has been shown (18) in screening assays for oxygen consumption and antigoiter activity on a series of 2'-alkyl-3,5-diiodo-DL-thyronines that the importance of steric factors may be demonstrated when the perpendicular nature of the planes of the phenyl rings has been fixed by sterically preventing their free rotations. A bulky 2'-substituent (Fig. 2), such as methyl or isopropyl, to provide a minimal interaction with the 3,5-iiodines and inner ring, must occupy a position distal in space to the inner ring. In such an oriented 2'-alkyl-3,5-diiodothyronine, a 3'-substituent must also be in the distal position, and a 5'-substituent must be in the proximal position. It has been observed (19–21) that the methyl group may function in place of 3' and 5' iodine atoms in the outer ring of thyroxine analogues. To provide a base for comparison with the
present series, 3’ methyl 3,5 diido l-thyronine has been tested in the antigoiter assay. For ease of synthesis and because of the stability of such o-methyl phenols, analogues possessing methyl groups in the 3’- or 5’-positions, sterically fixed by 2’-alkyl substitution, have been used to test steric requirements for outer ring substituents.

Differences in activity for the 2’,3’-dimethyl and 2’,5’-di-
methyl analogues of 3,5-diido-m-thyronine in the rat antigoiter assay have been attributed to a more favorable orientation in the distal position for the 3’-methyl group in the 2’,3’-dimethyl analogue (18). Such a difference could not previously be shown in the 3-day oxygen consumption assay, and the isomers have now been further examined together with related 2’-substituted thyronines.

The supporting evidence for the 4’- (or 2’-) hydroxyl requirement (22) has been largely inferential and based on the inactivity of “meta”-thyroxine (10,23) and on the generally reduced activity of alkyl and acyl derivatives of the 4’-hydroxyl group (2). By far the greatest number of analogues that have been evaluated have possessed the free 4’-hydroxyl group, and among these there have been the most active compounds, pointing to the importance of this function for thyromimetic activity. Many analogues in which the 4’-hydroxyl group has been masked by a group such as O-acetyl, O-methyl, O-phosphoryl, and O-carboxymethyl have shown thyroxine-like activity. However, no inactive 4’-hydroxylated analogue is known which becomes active when masked, and all active 4’-hydroxylated analogues are more potent than their masked counterparts in mammals (24-26).

These groups may presumably be removed in vivo, generating the free 4’-hydroxyl group.

Several antagonists to thyroxine have been found among compounds which lack substitution at position 4’. Barnes et al. (16) found that 3,5-diido-4-(3’-methyl-5’-ethylphenoxy)-l-phenylalanine was active as an antagonist in the calorigenic assay, and the corresponding 2’,5’-dimethyl, 2’-isopropyl, and 2’-isopropyl-5’-methyl derivatives were shown (18) to act as antagonists to thyroxine in the antigoiter assay. Of the few 4’-unsubstituted analogues tested for thyromimetic activity, only one, the 2’,3’-
dimethylphenoxy analogue, has been reported (18) to show thyroxine-like activity in rat antigoiter and oxygen consumption assays. The thyromimetic activity of this analogue has been studied further, and additional 4’-deoxyphenoxy analogues have been tested for both thyroxine-like and antagonistic activity.

Metabolic 4’-hydroxylation would be unlikely to occur in analogues in which the 4’-position is occupied by substituents other than hydroxyl, hydrogen, or masked hydroxyl groups. Previously reported analogues of this type include the O-4’-hydroxyphenyl- and O-4’-hydroxy-3’,5’-diiodophenyl ethers of thyroxine (27), and “meta”-thyroxine (23). These compounds were inactive when tested as thyrerimetics, and have not been reported or tested as antagonists. Two 4’ chlorophenoxy analogues were found devoid of either thyroxine-like or antagonistic properties (18). 3,5-Diido-4-(2’,4’-dimethylphenoxy)-l-phenylalanine was inactive as a thyromimetic, and active as an antagonist in the rat antigoiter assay (18). The corresponding 3’,4’-dimethyl analogue has been reported as an antagonist in the mouse oxygen consumption assay (16), and has been tested as an antagonist in the rat antigoiter assay in the present study.

The lack of thyroxine-like activity in the aliphatic and alicyclic series and the finding of both antagonistic and thyromimetic activity within a series of analogues of which the outer ring was an alkyl-substituted phenyl or even simple phenyl ring (see “Results and Discussion”), lead to the question of whether aromatic systems other than the benzenoid could be substituted for the outer ring of thyroxine. The 1-naphthyl (Fig. 3a) and 2-naphthyl (Fig. 3b) ring systems were especially attractive since they could provide structural analogues of the thyromimetic 2,3-dimethylphenyl ether (Fig. 3c) and the antagonistic 3,4-dimethylphenyl ether (Fig. 3d). The 4-hydroxy-1-naphthyl ether (3,5-diido-3,4-dialkylthyrone) (6), Fig. 3e) was of interest for comparison with the nonhydroxylated 1-naphthyl ether, and with the potent thyromimetic 2,3-dimethyl-4-hydroxyphenyl ether (Fig. 3e). The O-methyl ether (as its N-acetyl derivative (Fig. 3d)) of 3,5-diido-4-naphthyronine was included to provide information on a masked 4’-hydroxyl group in this series; the 3’-bromo derivative of 3,5-diido-4-naphthyronine (Fig. 3h) as a halogenated derivative in which the halogen substituent would occupy a relatively fixed position in space with respect to the rest of this sterically oriented molecule.

EXPERIMENTAL PROCEDURE

The antigoiter assay was based on that of Dempsey and Astwood (28) and was designed to detect activity relative to L-thyroxine of 0.2% or higher. Three-day oxygen consumption studies were considered useful in determining presence or absence of activity; the 10-day oxygen consumption assay was used to define the level of activity. The cholesterol-lowering method was considered to be a screening method and was consistent in indicating high and low activity, or the absence of activity at the levels tested. A summary of results for all compounds tested is presented in Table I.

Antigoiter Assay—Groups of five or six Long-Evans strain male rats weighing initially 125 ± 25 g were fed powdered Simonsen rat food either alone (normal controls) or containing 0.3% thiouracil. After 1 day on this regime, daily subcutaneous injections of the compounds in 50% aqueous polyethylene glycol (Carbowax 4000), in ethanolic-propylene glycol (10:90, volume for volume), propylene glycol, or 0.9% aqueous sodium chloride containing 0.01% sodium hydroxide, were administered for 10 days. On the 11th day, the rats were killed by chloroform

FIG. 1

FIG. 2

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inhalation, and the thyroid glands were excised, cleaned under the dissecting microscope, and weighed to the nearest 0.1 mg. Rats treated with vehicle alone and others treated with L-thyroxine served as controls. Potential antagonists were tested by injecting daily for 10 days an amount of L-thyroxine sufficient to cause significant reversal of the goitrogenic effect of thiouracil (usually 3.0 µg of L-thyroxine per 100 g of body weight) and concurrently, a 200-fold excess of the DL analogue. A thyroid weight significantly larger (at the 90% level of confidence) than that of the thyroxine-treated control was taken to indicate an antagonistic effect.

Compounds were initially tested for thyroxine-like activity at a molar ratio to an effective dose of L-thyroxine of 200:1. Those producing a maximal reduction in thyroid weight were tested at reduced dose levels in subsequent assays until a partial reversal of the goitrogenic effect was obtained. This was compared with the effect produced by the thyroxine standards in the same assay to estimate activity.

Oxygen Consumption Determination—Groups of five to seven adult male Sprague-Dawley rats weighing approximately 250 to 275 g and maintained on Purina dog chow and tap water were treated subcutaneously once daily for either 3 or 10 days. The potential thyromimetic agent was administered in a vehicle of 0.9% NaCl solution (pH 8.5 to 9.0). Experiments on rats treated with NaCl solution and L-triiodothyronine were run concurrently. After a 1-hour fast, oxygen consumption was determined on either the 4th or 11th day according to the method of Holtkamp et al. (29). A positive response was one in which an elevation of oxygen consumption was statistically significant at the 95% level of confidence over control values. Log dose-versus-response curves were plotted and activity estimated by the parallel line method.

Plasma Cholesterol Test—Groups of 7 to 10 intact male Sprague-Dawley rats weighing 275 to 300 g were maintained on Purina laboratory chow and tap water ad libitum. Prior to the start of treatment, the animals were fasted for 18 hours, at which time they were weighed and bled from the tail. The blood was withdrawn into heparinized hematocrit tubes. Total plasma cholesterol was determined by a modification of the method of Anderson and Keys (30). Subcutaneous treatment with the test agent was then started at a dose of either 1.5 or 15 µg per kg per day. This dose was continued for 7 days, whereupon blood was again withdrawn after an 18-hour fast. The dose level was then increased 10-fold and injections were continued for an additional 7 days. Every subsequent 7 days, the dosage was increased 10-fold until a dose of 1500 µg per kg per day was reached. Experiments with L-triiodothyronine-treated rats as well as with rats receiving the vehicle of 0.9% alkaline NaCl solution subcutaneously were run concurrently as controls.

RESULTS AND DISCUSSION

Aliphatic and Alicyclic Ethers of 3,5-Diiodotyrosine. The Requirement for Aromatic Character—In spite of a variety of polar, nonpolar, aliphatic, and alicyclic substitutions, all non-aromatic ethers of 3,5-diiodotyrosine tested proved to be completely without thyroxine-like activity in the antigoiter, oxygen consumption, and blood cholesterol assays (Table I). None of the 14 previously known alkyl-aryl or aralkyl-aryl ethers which may be considered as related analogues (2) displays a well-defined thyroxine-like activity in a variety of test methods. 3,5-Diiodo-4-isopropoxyphenylacetic acid, a compound recently tested to establish the requirement for the entire biphenyl ether system, was reported inactive in the blood cholesterol assay (31). On the basis of the low activity in mammals previously reported for related alkyl and aralkyl ethers (7, 13-17), and the by guest on October 20, 2017 http://www.jbc.org/ Downloaded from
TABLE I
Summary of tests for thyroxine-like and thyroxine-antagonistic activity

![Chemical structure](attachment:image.png)

<table>
<thead>
<tr>
<th>Compound administered</th>
<th>Antigoiter</th>
<th>Oxygen consumption</th>
<th>Plasma cholesterol activity</th>
</tr>
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<tr>
<td></td>
<td>R</td>
<td>R'</td>
<td>Dose(^a)</td>
</tr>
<tr>
<td>Isomer</td>
<td>R</td>
<td>R'</td>
<td>mg</td>
</tr>
<tr>
<td><strong>Aliphatic and alicyclic ethers</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1. (CH___________________________COOH</td>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL(^d) (CH___________________________COOH</td>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL(^d) (CH___________________________COOH</td>
<td>H</td>
<td>5.2</td>
<td>-</td>
</tr>
<tr>
<td>DL(^d) (CH___________________________COOH</td>
<td>H</td>
<td>4.8</td>
<td>-</td>
</tr>
<tr>
<td>DL(^d) CH___________________________COOH</td>
<td>H</td>
<td>4.6</td>
<td>-</td>
</tr>
<tr>
<td>DL(^d) (CH___________________________COOH</td>
<td>H</td>
<td>4.9</td>
<td>-</td>
</tr>
<tr>
<td>DL Cyclohexyl</td>
<td>COOH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL(^d) 3-Cyclohexenyl</td>
<td>COOH</td>
<td>5.2</td>
<td>-</td>
</tr>
<tr>
<td>DL(^d) 6-Hydroxy-3-cyclohexenyl</td>
<td>COOH</td>
<td>5.3</td>
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<td></td>
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<tr>
<td>DL(^d) Phenyl</td>
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</tr>
<tr>
<td>DL* Phenyl</td>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL(^d) 2-Methylphenyl</td>
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<td>4.3</td>
<td>-</td>
</tr>
<tr>
<td>DL(^d) 2,3-Dimethylphenyl</td>
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<td>8.3</td>
<td>+</td>
</tr>
<tr>
<td>DL* 2,4-Dimethylphenyl</td>
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<td>4.8</td>
<td>-</td>
</tr>
<tr>
<td>DL* 2,5-Dimethylphenyl</td>
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<td>4.2</td>
<td>-</td>
</tr>
<tr>
<td>DL* 3,4-Dimethylphenyl</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>DL* 3,5-Dimethylphenyl</td>
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<td>+</td>
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<tr>
<td>DL* 2-Isopropylphenyl</td>
<td>H</td>
<td>4.2</td>
<td>-</td>
</tr>
<tr>
<td>DL* 2 Isopropyl 5-methylphenyl</td>
<td>H</td>
<td>4.2</td>
<td>-</td>
</tr>
<tr>
<td>DL* 2,3-Dimethyl-4-chlorophenyl</td>
<td>H</td>
<td>4.9</td>
<td>-</td>
</tr>
<tr>
<td>DL* 2,5-Dimethyl-4-chlorophenyl</td>
<td>H</td>
<td>4.9</td>
<td>-</td>
</tr>
<tr>
<td><strong>4-Hydroxyphenyl ethers</strong></td>
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<td>+</td>
</tr>
<tr>
<td>DL 2-Methyl-4-hydroxyphenyl</td>
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</tr>
<tr>
<td>L 3-Methyl-4-hydroxyphenyl</td>
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<td>0.26</td>
<td>+</td>
</tr>
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<tr>
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<td>H</td>
<td>0.015</td>
<td>+</td>
</tr>
<tr>
<td>D 2,3-Dimethyl-4-hydroxyphenyl</td>
<td>H</td>
<td>2.3</td>
<td>+</td>
</tr>
<tr>
<td>DL 2,5-Dimethyl-4-hydroxyphenyl</td>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL 2-Methyl-4-hydroxy-5-iodophenyl</td>
<td>H</td>
<td>0.50</td>
<td>+</td>
</tr>
<tr>
<td>DL 2-Isopropyl-4-hydroxy-5-iodophenyl</td>
<td>H</td>
<td>2.2</td>
<td>+</td>
</tr>
<tr>
<td>DL 2-Isopropyl-4-hydroxy-5-methylphenyl</td>
<td>H</td>
<td>2.2</td>
<td>+</td>
</tr>
<tr>
<td><strong>Naphthyl ethers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL(^d) 2-Naphthyl</td>
<td>H</td>
<td>6.5</td>
<td>+</td>
</tr>
<tr>
<td>DL(^d) 1-Naphthyl</td>
<td>H</td>
<td>0.62</td>
<td>+</td>
</tr>
<tr>
<td>DL 4-Hydroxy-1-naphthyl</td>
<td>H</td>
<td>0.013</td>
<td>+</td>
</tr>
<tr>
<td>L 1 Hydroxy 1 naphthyl</td>
<td>H</td>
<td>0.013</td>
<td>+</td>
</tr>
<tr>
<td>DL 3-Bromo-4-hydroxy-1-naphthyl</td>
<td>H</td>
<td>0.085</td>
<td>+</td>
</tr>
<tr>
<td>DL 4-Methoxy-1-naphthyl</td>
<td>COOH</td>
<td>0.58</td>
<td>+</td>
</tr>
</tbody>
</table>

\(^a\) Maximal dose tested, or effective dose in milligrams per kg of body weight per day.

\(^b\) + = significantly different from control values \((p < 0.05)\); ± = borderline \((0.05 < p < 0.10)\); − = not significantly different from control values. (Continued.)
observation that diaryl ether analogues may act as antagonists (18) at a molar ratio to thyroxine at which the present aliphatic and alicyclic ether series was inactive, it is concluded that aromatic character in the outer ring position is not required for thyroxine analogues to act as antagonists in mammals; however, this feature seems to contribute to maximal activity.

Steric Relationships between Inner Ring and Substituents on Outer Ring of 2'-Alkyl-3,5-diiodothyronines—3'-Methyl-3,5-diiodo-L-thyronine was found to be 75% as active as L-thyroxine in the rat antigoiter assay. This figure is considerably lower than the activity of 250% of L-thyroxine reported for this compound in the oxygen consumption assay in thyroidectomized rats (21). However, in either case the methyl group may replace the iodine atom in the outer ring with retention of significant thyroxine-like activity, and conclusions may be drawn with regard to either a sterically oriented methyl group or halogen atom in the 3’- (or 5’-) position.

In the 10-day oxygen consumption test, 2’,3’-dimethyl-3,5-diiodo-L-thyronine, with its 3’-methyl group oriented in the distal position in space with respect to the inner ring, was found to be 13% as active as L-triiodothyronine, and approximately 65 times as potent as its 2’,5’-dimethyl isomer, which possesses a proximally oriented 5’-methyl group. In the rat antigoiter assay, the 2’,5’-dimethyl analogue showed approximately 50% of the activity of L-thyroxine, while the 2’,5’-dimethyl analogue was less than 1% as active (18), a difference of over 50-fold. The 2’,5’-dimethyl analogue was approximately 100 times as active as its 2’,5’-dimethyl isomer in the blood cholesterol test. The 2’,5’-dimethyl analogue showed the expected high activity in the three assays; the isomer was inactive at the highest dose tested in the blood cholesterol assay.

Since 2’,3’-dimethyl-3,5-diiodo-L-thyronine and 3’-methyl-3,5-diiodo-L-thyronine possess the same order of high activity, while the 2’,5’-dimethyl analogue is only 1 to 2% as active as these, the activating effect of the 3’-methyl group must be asso-
ciated with a steric positioning in the distal position (2’-methyl), or be capable of such orientation if steric orientation is not present (2’,6’-hydrogens). Differences in electronic (resonance and inductive) contributions by methyl groups of the 2’,3’-dimethyl and 2’,5’-dimethyl isomers would be expected to be negligible.

The related analogue 2’-isopropyl-5’-methyl-3,5-diiodo-DL-thyronine, which possesses a proximally oriented 5’-methyl group, has also been shown to possess a low order of activity in the three assays. A reevaluation of the antigoitrogenic activity (18) for 2’-methyl-3,5,5’-triiodo-DL-thyronine, in which all 3’-iodine substituents is proximally oriented, showed an activity of approximately 2% of L-thyroxine, a remarkably low figure for this close analogue of the potent thyroid hormone, 3,5,3’-triiodothyronine. The 2’-methyl-5’-ido compound displayed a correspondingly low activity in oxygen consumption and plasma cholesterol assays. These examples further illustrate that, for maximal thyroxine-like activity, activating substituents (e.g., halogen, alkyl) in the 3’-position should be either sterically oriented (2’-substitution) or capable of orienting themselves (2’,6’-hydrogens) distally in space with respect to the 3,5-diiodothyrosinyl inner ring.

4’-Hydroxyl Group as Requirement for Thyroxine-like Activity—The demonstration of a significant level of thyroxine-like activity for the 4’-deoxy analogue, 3,5-diiodo-4-(2’-3’-dimethylphenoxo)-DL-phenyalanine (18) (Table I) would seem to indicate a lack of requirement for the 4’-hydroxyl group for thyroxine-like activity. This is supported by the finding of thyroxine-like activity for the additional 4’-deoxy compounds, 3,5-diiodo-4-phenoxy-DL-phenyalanine and its 3’ isomer and for 3,5-diiodo-4-(3’,5’-dimethylphenoxo)-DL-phenyalanine. 3,5-Diiodo-4-phenoxy-DL-phenyalanine has recently been reported effective in lowering blood cholesterol (31).

However, a comparison of the relative activities of the thyr
osminic 4’-deoxy analogues with their 4’-hydroxylated counterparts (Table I) shows that the latter are always more potent. The only compound not available for direct comparison, 3’,5’-dimethyl-3,5-diiodo-DL-thyronine, has been reported to be 17% as active as L-thyroxine in the oxygen consumption assay (19). The enhanced activity for all 4’-hydroxy analogues, together with the observation of Stroud (32) that para-hydroxylation in vivo of diphenyl ethers can take place, raises the possibility that such metabolic hydroxylation by the organism is necessary before a 4’-deoxy analogue can exert its thyroxine-like action.

Bearing on this question is the observation that the 4’-deoxy analogues shown to act as thyroxine antagonists (18) have 4’-hydroxylated counterparts with a relatively low activity; less than 3% that of L-thyroxine in the antigoiter assay, and less than 1% of L-triiodothyronine in the 10-day oxygen consumption assay (Table I).

Therefore, it is postulated that metabolic 4’-hydroxylation may occur in the intact animal for those analogues possessing a free 4’-position, sufficient metabolite being formed to provide thyroxine-like activity, if that metabolite has an inherently high potency. Metabolic hydroxylation may also occur in the case of 4’-deoxy analogues that act as antagonists, but in this case the metabolite formed would possess too low an activity or be formed too slowly to overcome the antagonistic effect of the nonmetabolized deoxy compound. This antagonism is visualized as a competitive binding of antagonist with the biological receptor for thyroxine, with the antagonist lacking the requisite structural features (including the 4’-hydroxyl group) to perform the functional role of thyroxine.

All analogues tested to date in which the 4’-position is substi-
tuted by a group normally considered incapable of metabolic conversion to the hydroxyl group (e.g., halogen, methyl, phenoxo), unless capable of functioning in place of the hydroxyl (e.g., amino (1)), have been found to be inactive as thyromimetics, while some showed thyroxine-antagonistic properties. The 3’,4’-dimethylphenoxo analogue (Fig. 3f), previously shown to act as an antagonist in oxygen consumption studies (16), has
now been shown to possess thyroxine-antagonistic properties in the antigoiter assay, a property shared by the 2',4'-dimethyl isomer (18).

On the basis of available data, and with only the exception of a 2-naphthyl derivative to be discussed later, it would seem that a blocked 4'-position is incompatible with thyromimetic activity. It is, therefore, concluded that the 4'-hydroxyl group, a metabolic precursor to this, or its isosteric equivalent, is a structural requirement for thyroxine-like activity.

Nonbenzenoid Aromatic Rings—The 1-naphthyl ether of 3,5-diiodotyrosine (Fig. 3a) exhibited thyroxine-like activity in the three bioassay methods and is therefore a member of the small series of 4'-unsubstituted thyroxine analogues which shows such activity. Structurally and sterically, it may be compared with the 2',3'-dimethylphenoxy analogue (Fig. 3c), for in each case the substituents attached to the benzene ring must be oriented distally in space to the inner ring. The second aromatic ring of the 1-naphthyl group acts as a bulky orienting group in this case.

As was true for all other thyromimetic analogues unsubstituted in the 4'-position, the 4'-hydroxy derivative of the 1-naphthyl ether (3,5-diido-DL-naphthyronine, Fig. 3c), and its DL isomer, showed considerably higher thyroxine-like activity. In the rat antigoiter assay, this activity was as high or higher than that of L-thyroxine. The exact upper limit of activity is in some doubt, since assays performed with 3,5-diido-DL-naphthyronine dissolved in propylene glycol showed activity levels higher than L-thyroxine on a molar basis. Assays in which the L and DL isomers were dissolved in alkaline 0.9% NaCl solution, showed the L isomer to be equal in activity to L-thyroxine, and the DL isomer to possess a lower activity. Since the stability of 3,5-diiodonaphthyronine is poor in the presence of aqueous alkali (6), it is suspected that the lower assay values may be due to partial decomposition in the aqueous alkaline test solutions during the 10-day assay. Such instability in aqueous alkali has been observed with other thyroxine analogues (33). For the present, we assign to both 3,5-diido-DL-naphthyronine and to the DL isomer an activity equal to or greater than that of L-thyroxine in the rat antigoiter assay. In the 10-day oxygen consumption assay, activity for the DL isomer was estimated as 20% of L-triiodothyronine. The dose of 3,5-diido-DL-naphthyronine required to cause a significant depression of blood cholesterol was high for such an otherwise active compound.

The relatively high thyroxine-like activity for 3,5-diiodonaphthyronine could be accounted for in terms of several structural features. The presence of the 4'-hydroxyl group adds to evidence for the requirement of such a group at the biological site. The second aromatic ring of this naphthalene analogue contributes favorable characteristics, for 3,5-diido-DL-naphthyronine is at least 20 times as active as the simple phenolic analogue, 3,5-diido-DL-thyronine (6% L-thyroxine), and more active than the related alkyl-substituted compound, 3,5-diido-2',3'-dimethyl-DL-thyronine (50% L-thyroxine). It seems certain that the distally oriented second aromatic ring provides a favorable stereochemical feature, and of potential importance, additional aromatic character. It is speculative that this might relate to added opportunity for overlap of π-orbitals, either by receptor binding or by providing a more stable charge-transfer complex, or one with a lower barrier to energy transfer. Alternate possibilities include a contribution to a favorable oxidation-reduction potential to the outer ring, or greater stability to a transient free radical during electron transfer.

The additional naphthyl analogues serve to substantiate structure-activity relationships observed in the phenyl series. The high activity of 3,5-diido-DL-naphthyronine is reduced to about the level of the 4'-deoxy analogue, when it is converted to the 4'-O-methyl ether (Fig. 3d (N-acetyl); 5% L-thyroxine) derivative. This serves as an additional example of the requirement for an unmasked 4'-hydroxyl group for maximal activity. The reduced activity of the 3'-bromo analogue of 3,5-diido-DL-naphthyronine (Fig. 3f; 29% L-thyroxine) adds further data to substantiate the conclusion that higher activity is associated with analogues substituted at the distal 3'-position. Since 3,5-diido-DL-naphthyronine may be considered a distally 2',3'-substituted phenoxo analogue, substitution by bromine in the free proximal position ortho to the phenolic hydroxyl group results in reduced activity. In the examples studied, it would seem that a free 6'-proximal position is required for the highest level of thyromimetic activity.

Besides the fact that a nonbenzenoid aromatic ring can function in the outer ring position of thyroxine, the general features associated with maximal activity in the outer ring of 3,5-diiodothyronine are: a free hydroxyl group positioned 1,4 to an ether oxygen through an aromatic ring system; the additional bulk of the molecule oriented in the distal position with respect to the inner ring; and an unsubstituted hydrogen ortho to and on the proximal side of the 4'-hydroxyl group.

The 2-naphthoxy analogue (Fig. 3b) of this series shows a weak but reproducible activity in the rat antigoiter assay, although it was inactive in a 3-day screening test for effect on oxygen consumption. The activity found in the antigoiter assay would serve as evidence against a requirement for the 4'-hydroxyl group as a structural requirement, since the equivalent to this position in the 2-naphthy ring is blocked against metabolic hydroxylation. However, an alternate possibility may be considered. In this analogue, other positions in the naphthalene nucleus (e.g. position 6') are free for metabolic hydroxylation, and may then subsequently perform the normal function of a 4'-hydroxyl group since the 6-hydroxy-2-naphthyl ether also possesses hydroxyl and other oxygens conjugated through an aromatic system.

The synthesis and biological evaluation of isomeric hydroxynaphthyl ethers of 3,5-diiodotyrosine is under way, and will be reported in the near future.

Separation of Biological Effects by Structural Variation—The possibility exists that the initiating events for the various biological functions of the thyroid hormones are associated with different biological receptors, possessing related but varying...
minimal and optimal structural requirements. Alternatively, the same receptor and initiating event could be involved for all hormonal responses, but structural differences in analogues could lead to differences in distribution in the body, which could provide a dissociation of hormonal effects. In this study, no case has a complete separation of hormonal effects been observed. Those analogues showing relatively high activity in the oxygen consumption assay, also show high activity in the anti-goitrogen and cholesterol-lowering tests. There appear to be differences in order within these groups, however, which may provide a useful, if not absolute separation.

Hormone Receptor—A schematic representation of a receptor fitting the stereochemical requirements developed in this study, and interacting with L-triiodothyronine, is shown in Fig. 4. This hypothetical receptor is represented in three parts: a “binding receptor” constituted for binding to a 3,5-diiodotyrosil moiety, a “functional receptor” interacting with the outer ring and its substituents, and a “proximal steric block” to rationalize the generally reduced activity for proximally-3' substituted analogues. Compounds possessing requirements for binding to such a receptor, but lacking features related to the functional role of the hormone, have been shown to act as hormonal antagonists.

SUMMARY

A number of aliphatic, alicyclic, and aromatic ethers of 3,5-diiodothyrosine have been tested in the rat for their effects on thiouracil-induced goiter, oxygen consumption, and blood cholesterol levels. Within the present series, structural requirements for the outer ring of thyroxine were very similar, if not identical, for the various hormonal effects tested. These include: (a) aromatic character; (b) 4'-hydroxylation (or its isosteric equivalent) or the potential for metabolic hydroxylation in this position; (c) for maximal activity, an appropriate substituent (e.g. halogen, alkyl, aromatic ring), ortho (3') to the 4'-hydroxy group, and capable of positioning in space distal to the inner ring; (d) a free proximal 5'-position is required for the highest level of thyromimetic activity.

Three new thyromimetic analogues of thyroxine have been reported which are unsubstituted in position 4'. It has been shown that the 4'-hydroxyl analogues of each possesses a higher activity, and it is postulated that the deoxy analogues exert their activity following metabolic hydroxylation. 3,5-Diido-4-(3', 4'-dimethylphenoxy)-DL-phenylalanine has been observed to act as an antagonist to the antigoitrogenic effect of thyroxine in the rat. Antagonistic activity is related, in this case, to the blocked 4'-position.

The naphthalene ring system has been shown to be capable of replacing the benzene outer ring of thyroxine, and one analogue of this series, 3,5-diodonaphthyronine, has been shown to possess an activity equal to or greater than that of L-thyroxine. A hypothetical thyroid hormone receptor fitting the steric requirements described in this study has been represented schematically.

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REFERENCES

Thyroxine Analogues: VII. ANTIGOITROGENIC, CALORIGENIC, AND HYPOCHOLESTEREMIC ACTIVITIES OF SOME ALIPHATIC, ALICYCLIC, AND AROMATIC ETHERS OF 3,5-DIODO-TYROSINE IN THE RAT

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