Enzymic Conversion of Iodinated Thyronines to Iodinated Thyroacetic Acids*  
KENNICHI TOMITA AND HENRY A. LARDY  
From the Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin  
(Received for publication, July 11, 1960)

A previous paper (1) from these laboratories described the enzymic conversion of thyroxine and triiodothyronine to their corresponding acetic acid analogues. The enzyme system responsible for the degradation of the alanine side chain of these compounds was found in the mitochondrial fraction of rat kidney homogenate. It has since been shown to be most active in kidney, but to occur also in the mitochondria of liver (2, 3), heart muscle (2), and brain (2, 4).

Further examination of the specificity of this enzyme system has shown that in addition to thyroxine and triiodothyronine, 3,5 diiodothyronine, 3'-iodothyronine, and even thyronine are converted to their corresponding thyroacetic acids. This indicates that the enzyme system is not specific for the substituents on the thyronine rings. The main problem which remained was to determine the pathway by which the thyronines are converted to thyroacetic analogues. Attempts to identify intermediates on this pathway have been unsuccessful, and the synthesis of possible intermediates was therefore undertaken. The biological activity and metabolic conversions of these intermediates are presented in this paper.

EXPERIMENTAL PROCEDURE

Incubation, extraction, and chromatographic techniques were similar to those described before (1). The incubation mixture consisted of 5 ml of 0.1 M phosphate buffer, pH 7.4, 25 μmoles of DPN, and 15 ml of sonically treated rat (Sprague-Dawley, male, 200 to 250 g) kidney mitochondria. From 1 to 2 mg of the substrates were employed. This amount of thyroethanols was dissolved in 0.5 to 1 ml of 50% propyleneglycol; the thyronines were dissolved in 0.5 ml of 0.2 N Na2CO3. The incubation was carried out in 250-ml Erlenmeyer flasks for 2 hours at 37°. After the reaction mixture was extracted with 10 volumes of n-butanol-concentrated NH4OH (50:1, volume for volume), the concentrated crude material was chromatographed on Whatman No. 3MM paper (descending, about 15 hours at room temperature) with tertiary amyl alcohol saturated with 6 NH4OH as solvent. The compounds were located on a strip cut from the dried paper by spraying with 4-aminoantipyrine reagent (5).

For purification of radioactive triiodothyroethanol, after chromatography, the dry paper was cut into 1-cm-wide pieces and the activity was counted with a Geiger counter. The main product was recrchromatographed as above.

Commercially available materials were from the following sources: DPN, Pabst Laboratories; 4-aminoantipyrine, Eastman Kodak; p-methoxyphenylacetic acid, Matheson Company; DL-thyronine, Nutritional Biochemical Corporation; 3,5-diiodo-DL-thyronine, Hoffmann-LaRoche; and 3,5,3'-triiodothyroacetic acid (Triac), Smith, Kline and French Laboratories. Other compounds were prepared by established methods: 3'-ido-DL-thyronine (6, 7), 3,5-diiodothyroacetic acid (Diac) (8).

The synthesis of thyroethanols, by adaptations of the principle of Charmers et al. (9, cf. also 10, 11) is presented later in this paper. The structure of diiodothyroethanol was confirmed by removing iodine from the molecule with hydrogen in the presence of Raney nickel. The product, thyroethanol, was identical with the alcohol obtained by the LiAlH4 reduction of diiodothyroacetic acid. Thyroacetic acid was prepared from triiodo- or diiodothyroacetic acid by the Raney nickel hydrogen reduction. Thyronamine was prepared by decarboxylation of thyronine.

RESULTS

Substrate Specificity of the Thyroxine Degrading System

It has already been shown (1) that the enzymes of kidney mitochondria convert both thyroxine and triiodothyronine to their corresponding acetic acid analogues. The alanine side chains of 3,5-diiodothyronine, 3'-iodothyronine and thyronine itself are also degraded enzymically to form acetic acid analogues as is shown in Table I. The enzyme reaction extracts chromatographed on paper contain two major components detected with 4-aminoantipyrine. The slower moving materials (Spot I) are the added substrates. In each case, Spot II corresponds in Rf to the expected thyroacetic acid. Since no 3'-iodothyroacetic acid was available for comparison, the metabolic product from 3'-iodothyronine was deiodinated by hydrogenation. The resulting deiodinated compound migrated at the same rate as thyroacetic acid. More rigorous proof of structure of these metabolic products was considered unnecessary in view of the detailed characterization of the acetic acid derivatives produced from thyroxine and triiodothyronine (1).

Pathway of Side-Chain Degradation

Two possible routes for the degradation of thyronines to thyroacetic acids are depicted in Fig. 1. Decarboxylation via Reaction 1-a could produce thyronamines which have thyroid hormone...
Table I

Enzymic conversion of thyronines and of thyronamine to thyroacetic acids

The values recorded are for two separately conducted experiments.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Spot I</th>
<th>Spot II</th>
<th>RF</th>
<th>Authentic thyroacetic acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,5-Diiodo-DL-thyronine</td>
<td>0.48</td>
<td>0.64</td>
<td>0.48</td>
<td>3,5-Diiodothyroacetic acid</td>
</tr>
<tr>
<td></td>
<td>0.48</td>
<td>0.67</td>
<td></td>
<td>0.65</td>
</tr>
<tr>
<td>3'-Iodo-DL-thyronine</td>
<td>0.20</td>
<td>0.22</td>
<td>0.48</td>
<td>Thyroacetic acid</td>
</tr>
<tr>
<td></td>
<td>0.22</td>
<td>0.30</td>
<td></td>
<td>0.48 (after hydrolysis)</td>
</tr>
<tr>
<td>DL-Thyronine</td>
<td>0.26</td>
<td>0.34</td>
<td></td>
<td>Thyroacetic acid</td>
</tr>
<tr>
<td></td>
<td>0.34</td>
<td>0.49</td>
<td></td>
<td>0.49</td>
</tr>
<tr>
<td>Thyronamine</td>
<td>0.36</td>
<td>0.49</td>
<td></td>
<td>0.49</td>
</tr>
</tbody>
</table>

Enzymic Conversion of Thyroethanols to Thyroacetic Acids

The next transformation in the scheme (Reaction 2) should yield iodinated thyroacetaldehydes. The evidence cited immediately above indicates that these carbonyl compounds are also not readily detected. Attempts to synthesize them proved unfruitful. Possible precursors—variously iodinated thyroethanols—were then synthesized as described below and were found to be converted to the corresponding thyroacetic acids (Table II). The parent thyroethanols all moved close to the front in the tertiary amyl alcohol-6-NH₂OH solvent system. The relative mobilities of the rechromatographed products correspond to those of the authentic acetic acid analogues. The spots formed by mixtures of the product and the authentic acetic acids were not elongated as compared to either alone, attesting to their identity. I¹³ labeled triiodothyroethanol was converted to a radioactive product which migrated on paper at the same rate as triiodothyroacetic acid. No such product was produced when the experiment was conducted with a boiled enzyme solution. The product was eluted from the paper and recrystallized with authentic triiodothyroacetic acid. The specific activity (c.p.m. per 100 µg) on successive recrystallizations from methanol by addition of water were: original mixture, 253; 1st recrystallization, 280; 2nd, 285; 3rd, 275; 4th, 276.

These data clearly establish that thyroethanols are converted by the enzyme preparation to thyroacetic acids. Thyroacetaldehyde is formed by adduction of water.

Table II

Enzymic conversion of thyroethanols to thyroacetic acids

<table>
<thead>
<tr>
<th>Substrates</th>
<th>RF</th>
<th>Products</th>
<th>Products of thyroacetic acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroethanol</td>
<td>0.45</td>
<td>Thyroacetic acid, 0.47</td>
<td></td>
</tr>
<tr>
<td>3,5-Diiodothyroethanol</td>
<td>0.65</td>
<td>3,5-Diiodothyroacetic acid, 0.65</td>
<td></td>
</tr>
<tr>
<td>3,5,3'-Triiodothyroethanol</td>
<td>0.57</td>
<td>3,5,3'-Triiodothyroacetic acid, 0.56</td>
<td></td>
</tr>
</tbody>
</table>

Metabolism of Thyronines

**Synthesis of Triiodothyroethanol**

Ethyl 3-hydroxyphenylacetate (I)—p-Methoxyphenylacetic acid, 100 g, was refluxed with glacial acetic acid, 500 ml, and hydrobromic acid, 48%, 250 ml, instead of hydriodic acid (10), for 10 hours. The mixture was evaporated in a vacuum and the residue was recrystallized from 500 ml of hot water, yielding 64 to 80 g of 3-hydroxyphenylacetic acid, m.p. 149-151°. A mixture of the hydroxy acid, 150 g, absolute alcohol, 450 ml, and concentrated H₂SO₄, 8 ml, was refluxed for 3 hours. About 300 ml of alcohol was distilled off from the reaction mixture under reduced pressure at 40°. Addition of 600 ml of water separated the ester (lower layer). Ether extracts, 2 × 150 ml, of the aqueous layer were combined with the ester layer, washed with water, 5% NaHCO₃, and water, and dried over Na₂SO₄.

The ester distilled at 150-157° at 1.5 mm in a yield of 159 g (89.4% of the theory).

Reduction of (I) to p-Hydroxyphenethyl Alcohol (Tyrosol) (II)—Ethyl 3-hydroxyphenyl acetate was reduced to II with LiAlH₄, but it could be prepared in somewhat better yield by reduction with Na and n-butanol. The method used was similar to the one for preparation of oleyl alcohol from butyl oleate (19). In a 2-liter three-necked flask, equipped with two large reflux condensers, 90 g (0.5 mole) of ethyl 3-hydroxyphenylacetate were dissolved in 1 liter of anhydrous n-butanol, and 60 g (2.6 moles) of clean sodium cut in cubes were added at once. In 5 minutes, vigorous reaction started and the flask was cooled. After all the sodium had reacted, 55 ml of water were added and the mixture was acidified with concentrated HCl (about 250 ml). The butanol layer was separated and evaporated in a vacuum. The aqueous layer was extracted with ether (total 1 liter) and the ether solution was mixed with the butanol residue. After washing the solution with 5% NaHCO₃ and water, the ether extract was dried over Na₂SO₄ and evaporated. The crystalline residue was dissolved in hot CHCl₃, treated with charcoal and cooled to yield 53.7 g (77.8% of theory) of almost colorless needles, m.p. 89-91°. The reported m.p. is 90° (20).

Tyrosol Dibenzoate was prepared with benzoyl chloride in pyridine. Colorless fine needles, recrystallized from 95% alcohol, melted at 110-112°.

**Table 1**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁₂H₁₉O₃ (346.36)</td>
<td>C 76.28, H 5.23</td>
<td>C 76.48, H 5.35</td>
</tr>
<tr>
<td>p-Hydroxyphenethyl Acetate</td>
<td>C 76.28, H 5.23</td>
<td>C 76.48, H 5.35</td>
</tr>
<tr>
<td>(III)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosol Dibenzoate</td>
<td>C 76.28, H 5.23</td>
<td>C 76.48, H 5.35</td>
</tr>
<tr>
<td>(III)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ethyl 3-hydroxyphenylacetate was reduced to II with LiAlH₄, but it could be prepared in somewhat better yield by reduction with Na and n-butanol. The method used was similar to the one for preparation of oleyl alcohol from butyl oleate (19). In a 2-liter three-necked flask, equipped with two large reflux condensers, 90 g (0.5 mole) of ethyl 3-hydroxyphenylacetate were dissolved in 1 liter of anhydrous n-butanol, and 60 g (2.6 moles) of clean sodium cut in cubes were added at once. In 5 minutes, vigorous reaction started and the flask was cooled. After all the sodium had reacted, 55 ml of water were added and the mixture was acidified with concentrated HCl (about 250 ml). The butanol layer was separated and evaporated in a vacuum. The aqueous layer was extracted with ether (total 1 liter) and the ether solution was mixed with the butanol residue. After washing the solution with 5% NaHCO₃ and water, the ether extract was dried over Na₂SO₄ and evaporated. The crystalline residue was dissolved in hot CHCl₃, treated with charcoal and cooled to yield 53.7 g (77.8% of theory) of almost colorless needles, m.p. 89-91°. The reported m.p. is 90° (20).

Tyrosol Dibenzoate was prepared with benzoyl chloride in pyridine. Colorless fine needles, recrystallized from 95% alcohol, melted at 110-112°.

**Table 1**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁₂H₁₉O₃ (346.36)</td>
<td>C 76.28, H 5.23</td>
<td>C 76.48, H 5.35</td>
</tr>
<tr>
<td>p-Hydroxyphenethyl Acetate</td>
<td>C 76.28, H 5.23</td>
<td>C 76.48, H 5.35</td>
</tr>
<tr>
<td>(III)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosol Dibenzoate</td>
<td>C 76.28, H 5.23</td>
<td>C 76.48, H 5.35</td>
</tr>
<tr>
<td>(III)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4. The precipitate was collected and recrystallized twice from dilute acetic acid; yield, 38.2 g (70.8% of theory), m.p. 91.5–92.5°.

**C₇H₆O₃N₂** (270.20)
Calculated: C 44.44, H 3.73, N 10.36
Found: C 44.58, H 3.57, N 10.43

**Dinitrotyrosol Diacetate**—A mixture of IV (2.5 g) and 10 ml of acetic anhydride was heated with one drop of concentrated H₂SO₄ for 3 hours at 100 110°. The dark reddish solution was diluted with 50 ml of ice water and left at room temperature overnight. The light brown crystalline mass, after being washed with cold water, was recrystallized from glacial acetic acid (about 5 ml) three times, yielding light yellow prisms which melted at 90–92°.

**C₆H₆O₃N₂** (312.2)
Calculated: C 46.18, H 3.87, N 8.97
Found: C 46.29, H 3.69, N 9.23

β-[3,5-Diiodo-4-(4'-methoxyphenoxy)phenyl]-ethyl Acetate (V)—A mixture of IV, 10.8 g (40 mMoles), and p-toluene sulfonfyl chloride, 8.4 g (44 mMoles), in dry pyridine, 16 ml, was heated in an oil bath (100–105°) for 30 minutes. p-Methoxyphenol, 14.9 g (120 mMoles) was added, and the solution was heated in an oil bath (100–105°) for 30 minutes. The reaction mixture was taken up in 75 ml of CHCl₃ and washed successively with three 100 ml portions each of 2 N HCl, 2 N NaOH, and water. The CHCl₃ extract was dried over Na₂SO₄ and evaporated in a vacuum. The dark crystalline residue, 11.4 g, was taken up in 200 ml of dry benzene and passed through an alumina column (2 × 10 cm). The eluate was collected until it contained a negligible residue on evaporation (total, 350 ml). The crystalline residue (10.1 g) was recrystallized from glacial acetic acid, yielding yellow prisms; m.p. 110–111.5°.

**C₅H₆O₃N₂** (376.31)
Calculated: C 54.25, H 4.28, N 7.48
Found: C 54.20, H 4.42, N 7.48

p-Toluene sulfonfyl chloride could be replaced with methane sulfonfyl chloride, 5.27 g (46 mMoles). The reaction mixture was refluxed for 10 minutes before adding p-methoxyphenol and for 20 minutes after the addition. The yield was about the same.

β-[3,5-Dinitro-4-(4'-methoxyphenoxy)phenyl]-ethyl Alcohol—Dry HCl gas was passed for 30 minutes through an ice-cold suspension of 2 g of the acetate (V) in 75 ml of absolute ethanol. Crystals went into solution slowly. The mixture was refluxed for 4 hours and the solvent was distilled. The yellow crystalline residue was recrystallized from dilute ethanol and then from 95% alcohol, 10 ml, to yield 1.59 g (90% of theory) of yellow prisms; m.p. 137–139°.

**C₂H₆O₃N₂** (334.28)
Calculated: C 53.89, H 4.28, N 8.38
Found: C 54.05, H 4.15, N 8.69

Acetylation of the 3,5-dinitro-4-(4'-methoxyphenoxy)phenyl-ethyl alcohol thus obtained with glacial acetic acid and HzSO₄ gave a product identical to V, thus establishing the nature of the side chain.

**β-[N,N'-Diacetyl-3,5-diamino-1-(4'-methoxyphenoxy)phenyl]-ethyl Acetate (VI)**—Dinitro compound (V), 1 g, suspended in 100 ml of glacial acetic acid was hydrogenated in the presence of 0.1 g of 10% Pd on charcoal at room temperature (1 to 2 hours). The filtered solution was evaporated to dryness in a vacuum at 40°. The residue was heated with 30 ml of acetic anhydride at 70–80° for 3 hours. After the excess of reagent was decomposed with water, the reaction mixture was evaporated in a vacuum. The residue was recrystallized three times from dilute acetic acid after being treated with charcoal. Yield, 310 mg of colorless needles, m.p. 162–164°.

**C₆H₆O₃N₂** (400.4)
Calculated: C 63.33, H 6.04, N 6.99
Found: C 63.23, H 5.97, N 7.21

β-[3,5-Diiodo-4-(4'-methoxyphenoxy)phenyl]-ethyl Acetate (VII) was refluxed for 7 hours with 100 ml of glacial acetic acid (100 ml) was hydrogenated in the presence of 0.4 g of 10% Pd on charcoal. The crystals went into solution as hydrogenation proceeded. The theoretical amount of hydrogen was consumed in 3 to 5 hours. The reaction mixture was filtered through a sintered glass funnel into ice-cold, magnetically stirred, concentrated H₂SO₄ (50 ml). The color of the filtrate (diamine) was from almost colorless to light blue.

**Tetratization**—NaNO₂ (5 g) was suspended in ice-cold concentrated H₂SO₄ (40 ml) and warmed carefully with shaking to make a clear, pale yellow solution which was placed in a 1-liter three-necked flask equipped with a thermometer, a dropping funnel, and a powerful stirrer. The flask was placed in an ethanol bath (0 to −5°) and glacial acetic acid (80 ml) was added slowly with rapid stirring to form a thick white suspension. After the bath temperature was lowered to −20°, the filtered hydrogenation mixture was added dropwise over 2 hours. The temperature of the contents was kept below −10°. Stirring of the redish brown tetrazo solution was continued for an additional hour.

**Iodination**—A mixture of 27 g of NaI, 15.2 g of I₂, and 500 ml of water was stirred for several hours and, before the iodination, 3.6 g of urea and 200 ml of CHCl₃ were added. The tetratol solution was poured into the iodination mixture through a cold funnel during 5 minutes and the stirring was continued for 1 to 2 hours at room temperature. The chloroform layer was separated, and the aqueous layer was extracted several times with 150 ml portions of CHCl₃. The combined CHCl₃ extracts were washed with water, 5% sodium metabisulfite, water again, and dried over MgSO₄. After evaporation of the solvent, the dark crystalline residue (10 to 12.5 g) was taken up in benzene (75 ml) and passed through an alumina column (2 × 9 cm). Most of the diiodo compound (6.1 to 10.8 g) came out with the first 100 ml of eluate. One recrystallization from glacial acetic acid (20 ml, charcoal) produced 7.3 to 8.5 g (67–78% of theory) of almost colorless prisms melting at 106–108°. For analysis, the compound was recrystallized three times; m.p. 108–110°.

**C₇H₆O₃N₃I₃** (588.14)
Calculated: C 47.94, H 2.96, I 47.17
Found: C 47.23, H 2.92, I 47.50

β-[3,5-Diiodo-4-(4'-hydroxyphenoxy)phenyl]-ethyl iodide (VIII) was refluxed for 7 hours with 100 ml of glacial acetic acid (100 ml) of freshly redistilled HI (b.p. 125–126°) in the
presence of 1 g of red phosphorus. The clear red solution was decanted, the phosphorus was washed with water three times, and the cloudy washings (about 500 ml) were mixed with the decanted solution. After the mixture was cooled overnight, the yellow precipitate was collected, washed with water, and recrystallized from 30 ml of glacial acetic acid (charcoal) to yield 10 g (91% of theory) of almost colorless needles, m.p. 164-166°. The analytical sample was recrystallized several times from the same solvent or from absolute alcohol; m.p. 166-168°.

**C₄H₆O₃I₂ (391.99)**

Calculated: C 28.40, H 1.87, I 64.31
Found: C 28.51, H 1.98, I 64.70

This compound gave positive phenol tests with Folin-Dennis and 4-aminophenylpyrpyrine reagents. With 2% ethanolic AgNO₃ solution it produced precipitates which were not soluble in 5% HNO₃.

Two grams of VIII were dissolved in warm ethanol (30 ml) and mixed with 30 ml of 2 N NaOH. After 2 hours at room temperature, the reaction mixture was slightly acidified with concentrated HCl, diluted further with water, and cooled overnight. The white precipitate was recrystallized once from dilute ethanol and then twice from Skelly C. Colorless clustered needles, m.p. 123-125°, gave positive phenol tests, but a negative halogen test. The product was probably 3,5-diido-4-(4'-hydroxyphenoxystyrene).

The iodide (VIII), 5.92 g (0.01 mole), was dissolved in glacial ethanol and then twice from charcoal. Colorless clustered needles, m.p. 140-145°, was obtained. Benzene seemed better for further purification, but attempts to prepare tetraiodothyroethanol in a similar way with 4 atom equivalents of iodine were also made, and fine needles, melting around 200°, were obtained. On repeated recrystallization from alcohol, however, the solution gradually became pinkish-yellow, indicating decomposition.

**β-[3,5-Diido-4-(4'-hydroxyphenoxo)]phenyl]ethyl Acetate (IX)**

—The iodide (VIII), 5.92 g (0.01 mole), was dissolved in glacial acetic acid, 750 ml; dried silver acetate, 3.34 g (0.02 mole), was added and the mixture was stirred for 1 hour at room temperature and for 3 hours in a boiling water bath. The silver salts were separated by filtration and the filtrate was evaporated to dryness in a vacuum (40°). The dark residue was extracted three times with 150 ml portions of CHCl₃; the combined extracts were filtered and evaporated to dryness in a vacuum.

The reddish brown crude crystals were recrystallized from benzene (charcoal) to yield 4.5 g of almost colorless needles, which softened at 80-90°, resolidified over 100°, and melted at 140-145°. Several more recrystallizations from benzene raised the final melting point to 146-148°. The compound gave positive phenol tests and a negative test with AgNO₃.

**C₁₂H₁₆O₁₁I₂ (608.0)**

Calculated: C 35.03, H 2.64, I 52.40
Found: C 35.03, H 2.64, I 52.40

Attempts to prepare tetraiodothyroethanol in a similar way with 4 atom equivalents of iodine were also made, and fine needles, melting around 200°, were obtained. On repeated recrystallization from alcohol, however, the solution gradually became pinkish-yellow, indicating decomposition.

**3,5,3'-Triiodothyroethanol (XI)**—Diodothyroethanol, 964 mg (2 mmoles), was dissolved in a mixture of ethanol, 145 ml, and concentrated NH₄OH, 48 ml. The reaction vessel was cooled in ice and 4 ml of 1 N HCl solution was added dropwise while stirring the solution continuously. After 1 hour, the colorless reaction mixture was evaporated in a vacuum (40°). The residue was dissolved in 60 ml of boiling ethanol, diluted with 100 ml of water, and cooled overnight. Repeated recrystallization from 50% alcohol raised the melting point to 173-175°, but the crystals sintered around 150°. Benzene seemed better for further purification, but the purified sample still showed sintering at 160-165° and finally melted at 186°.

**C₅H₆O₃I₃ (482.1)**

Calculated: C 34.87, H 2.50, I 52.65
Found: C 35.03, H 2.64, I 52.40

**Thyroethanol**—Preparation A: From 3,5-diiodothyroacetic acid—3,5-diiodothyroacetic acid, 1.5 g, was dissolved in a mixture of methanol, 2 ml, and concentrated NH₄OH, 2 ml. A cyclohexane solution of 150 μg of I₂ containing 100 mc I¹³¹ was added, and the mixture was agitated until the purple color disappeared. The reaction mixture was concentrated and the residue extracted twice with n-butanol. After being washed with water, the butanol extracts were evaporated to dryness in a vacuum. The residue was taken up in a small amount of alcohol and chromatographed on paper to remove the remaining iodide which stayed near the origin. The radioactivity near the solvent front migrated at a rate characteristic of triiodothyroethanol (Rₛ = 0.88). It was extracted from the paper and used as a substrate without further purification.

**3,5,5'-Triiodothyroethanol (X)**—One gm of the acetate (IX) was dissolved in 30 ml of ethanol and mixed with 30 ml of 2 N NaOH. Hydrolysis was carried out for 4 hours at room temperature. The yellow reaction mixture was first acidified with concentrated HCl, then diluted with water to about 100 ml and cooled overnight. The crystals were collected, washed with water, and recrystallized from dilute ethanol (charcoal). Colorless tiny needles (0.9 g) melted at 180-182° with sintering. Several recrystallizations from benzene raised the m.p. to 185-187.

**C₃H₄O₃I₅ (482.1)**

Calculated: C 34.87, H 2.50, I 52.65
Found: C 35.03, H 2.64, I 52.40

**Preparation B:** From 3,5-diodothyroethanol—3,5-diodothyroethanol, 40 mg, in a mixture of ethanol, 15 ml, and concentrated NH₄OH, 5 ml, was hydrogenated under ordinary pressure in the presence of Raney nickel, W-2 (21), (3 ml, about 1.5 g)
for several hours. The filtrate was evaporated to dryness in a vacuum and the residue recrystallized from dilute ethanol or benzene; the m.p. of 141–142° was not depressed by mixing the product with preparation A.

\[ C_{12}H_9O_3 (230.3) \]
Calculated: C 73.02, H 6.12
Found: C 73.00, H 6.12

The dibenzoate of thyroethanol prepared with benzoyl chloride and pyridine melted at 119–121° when crystallized from 95% ethanol.

\[ C_{12}H_{13}O_5 (438.5) \]
Calculated: C 76.69, H 5.13
Found: C 76.51, H 5.13

**Other Syntheses**

**Thyroacetic Acid** was prepared in 83% yield from commercial triiodothyroacetic acid by procedure B for thyroethanol. After evaporating the ethanol, the crystalline residue was taken up into 50 ml of boiling 0.1 N \( \text{Na}_2\text{CO}_3 \). The hot filtrate was acidified with concentrated \( \text{HCl} \) and cooled. The crystalline product was recrystallized from 30% acetic acid or boiling water to yield 650 mg of colorless needles; m.p. 189–191°.

\[ C_{14}H_{15}O_2N (229.3) \]
Calculated: C 73.53, H 6.61, N 6.34
Found: C 73.52, H 6.60, N 6.40

**Thyronamine** was prepared by decarboxylating commercial di-thyronine by heating it with diphenylamine under hydrogen (13). The product was recrystallized from water; m.p. 135–137°.

\[ C_{14}H_{15}O_2N (229.3) \]
Calculated: C 73.53, H 6.61, N 6.40
Found: C 73.52, H 6.60, N 6.34

**DISCUSSION**

The conversion of variously iodinated thyronines to corresponding thyroacetic acids is a natural process in extrathyroidal mammalian tissues. Roche and Michel et al. (22–25) have demonstrated the occurrence of triiodothyroacetic-1° acid in several tissues of thyroidectomized rats after the administration of labeled triiodothyronine. They too have found that the number of iodine atoms or their position on the thyronine rings is not critical (26, 27). In contrast to the report (28) that 3, 5, 3'-triiodo-\( \delta \)-thyronine is converted to triiodothyroacetic acid in the intact rat, it has been found that the kidney mitochondrial enzyme system does not convert \( \delta \)-thyronine to tetraiodothyroacetic acid (29). Thus, there may be additional routes for the degradation of the thyronines in the intact animal, but the combined evidence from several laboratories now points to Reactions 1b, 2, and 3 of Fig. 1 as the main pathway.

**SUMMARY**

An extract of rat kidney mitochondria, fortified with diphosphopyridine nucleotide, converts 3, 5-diiodothyronine, 3'-iodothyronine, and uniodinated thyronine, as well as thyroxine and triiodothyronine, to their corresponding acetic acid analogues.

Although thyronamine is converted to thyroacetic acid, iodinated thyronamines are not, indicating that they are not intermedias in the conversion of iodinated thyronines to acetic acid analogues.

Iodinated thyropropyric acids and thyroacetaldehydes could not be detected as intermediates, but synthetic diiodothyropropyric-2-C\(^{14}\) acid was converted to radioactive diiodothyroacetic acid.

The synthesis and biological activity of 3, 5, 3'-triiodo-, 3, 5-diiodo-, and mon-iodinated thyroethanols are described. These compounds were oxidized to their respective thyroacetic analogues by the kidney enzyme system.

It is concluded that the enzymic conversion of iodinated thyronines to iodinated thyroacetic acids proceeds by way of iodinated thyropropyric acids and thyroacetaldehydes.

**REFERENCES**

2. **Albright, E. C., Tomita, K., and Larson, F. C., Endocrinol., 64, 205 (1959).**
4. **Tata, J. R., Rall, J. L., and Rawson, R. W., Endocrinol., 60, 83 (1957).**
15. **Tomita, K., Federation Proc., 16, 400 (1957).**
17. **Yamamoto, K., J. Physiol., 9, 394 (1950).**
22. **Roche, J., Michel, K., Jouan, P., and Wolf, W., Endocrinol., 59, 425 (1956).**
23. **Roche, J., Michel, R., and Jouan, P., Compt. rend. soc. biol., 150, 629 (1956).**
24. **Michel, R., and Etling, N., Compt. rend. soc. biol., 151, 36 (1957).**
25. **Nataf, B., and Spez, M., Compt. rend. soc. biol., 152, 1337 (1958).**
27. **Roche, J., Michel, R., Nunez, J., and Jacqueulin, C., Compt. rend. soc. biol., 152, 1640 (1958).**
29. **Larson, F. C., Tomita, K., and Albright, F. C., Endocrinol., 65, 356 (1959).**
Enzymic Conversion of Iodinated Thyronines to Iodinated Thyroacetic Acids
Kenkichi Tomita and Henry A. Lardy


Access the most updated version of this article at http://www.jbc.org/content/235/11/3292.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/235/11/3292.citation.full.html#ref-list-1