Synthesis and Biological Activity of O-Methyl Derivatives of Thyroid Hormones*

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(Received for publication, June 26, 1961)

In 1938, Loeser, Ruland, and Trikojus (12) reported that the direct methylation of thyroxine with diazomethane produced the O-methyl derivative. In the case of 3,5-diiodothyronine and diiodotyrosine, however, both the phenolic and amino groups were methylated (and also some formation of the allyl side-chain derivative was observed1). In our experience, all thyroidine methyl esters were smoothly methylated with diazomethane in an ethanol-ether mixture to produce a satisfactory yield of O-methyl derivatives regardless of the number of iodine atoms on the molecule. A proof of the structures of the compounds isolated is summarized in Fig. 1.

O-Methyl thyronine prepared with diazomethane was identical in the following respects with the product obtained by reaction of N-benzoyl thyroxine with dimethyl sulfate followed by hydrolysis: (a) the melting points and infrared spectra of their hydrochlorides; (b) the infrared spectra of the free ethers and Dr. Hems' authentic sample, and (c) their paper chromatographic behavior in tert-amyl alcohol saturated with 6 N NH₄OH. The product obtained by refluxing O-methyl thyronine with HBr (48%) acetic acid (1:2, volume for volume) was also compared with authentic thyroxine; they had the same melting point and paper chromatographic behavior. In a similar way, the identity of O-methyl-3,5-diiodothyronine prepared by these two routes was established.

The iodinated thyronines (thyroxine, 3,5,3′-triiodothyronine, and 3,5-diiodothyronine) were allowed to react with diazomethane, but have since succeeded, for they have used this compound in biological experiments (15, 16).

We are indebted to Drs. C. M. Greenberg, A. E. Heming, and H. L. Saunders for these determinations.

† V. M. Trikojus, personal communication.
Fig. 1. Reaction sequence establishing the identity of O-methyl thyronines. OMe, O-methyl; T4, thyroxine; T3, triiodothyronine; T2, diiodothyronine.

Raney Ni. The products were identical with O-methyl thyronine with respect to melting points, paper chromatographic behavior and infrared spectra (Table I). It was therefore concluded that our main products prepared with diazomethane were not methylated on the amino group. Smaller amounts of N-methylated derivatives may have been formed during the synthesis and separated from the main product during the repeated crystallizations.

**Biological Activities**

The relative activities of these O-methyl ethers in different bioassays are summarized in Table II. O-Methylation did not diminish the high activities of thyroxine, triiodothyronine, and triiodothyroacetic acid on tadpole metamorphosis. The activities of diiodothyronine and O-methyl diiodothyronine were assayed only once and are not significantly different. Like its parent compound, O-methyl thyronine was not detectably active. In the rat goiter prevention assay, O-methylation destroyed virtually all of the activity of thyroxine (13), an appreciable part of triiodothyronine and none of triiodothyroacetic acid. In the basal metabolic rate assay with intact rats, O-methyl thyroxine was about one-third as effective as thyroxine and O-methyl triiodothyronine was about one-half as active as its parent compound. In a determination with thyroidectomized rats O-methyl triiodothyroacetic acid was about two-thirds as active as triiodothyroacetic acid.

The biological activities of O-methyl triiodothyronine and O-methyl triiodothyroacetic acid of industrial origin have been reported in the literature, although the synthesis and physical constants have not been reported previously. Our findings for

**Table I**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Route of preparation</th>
<th>M.P. (decomposition)</th>
<th>Deliodinated product</th>
<th>Demethylated product</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-Methyl-DL-thyronine</td>
<td>CH₃N₂</td>
<td>210–212°</td>
<td>206–208°</td>
<td>250–251°</td>
</tr>
<tr>
<td>O-Methyl-LE-thyronine</td>
<td>(CH₃)₄SO₄</td>
<td>210–212°</td>
<td>205–207°</td>
<td>0.50</td>
</tr>
<tr>
<td>O-Methyl 3,5-diiodo-DL-thyronine</td>
<td>CH₃N₂</td>
<td>218–220°</td>
<td>202–205°</td>
<td>0.50</td>
</tr>
<tr>
<td>O-Methyl 3,5-diiodo-LE-thyronine</td>
<td>(CH₃)₄SO₄</td>
<td>217–219°</td>
<td>201–203°</td>
<td>0.48</td>
</tr>
<tr>
<td>O-Methyl 3,3',5-triiodo-L-thyronine</td>
<td>CH₃N₂</td>
<td>212–214°</td>
<td>203–205°</td>
<td>0.49</td>
</tr>
<tr>
<td>O-Methyl-dL-thyroxine</td>
<td>CH₃N₂</td>
<td>224</td>
<td>203–205°</td>
<td>250–252°</td>
</tr>
</tbody>
</table>
TABLE II

Biological activities of O-methylated compounds

The activities of L-triiodothyronine are taken from (17), assuming the β isomer of thyroxine to be inactive. Values for O-methyl thyroxine are from (13).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Comparative activity (molar basis)</th>
<th>Metamorphosis of frog (days)</th>
<th>Goiter prevention (rat)</th>
<th>Basic metabolic rate (rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,5-Diiodo-L-thyronine</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>O-Methyl-3,5-diiodo-L-thyronine</td>
<td>0.5</td>
<td>0.3</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>L-Triiodothyronine</td>
<td>10–40</td>
<td>20</td>
<td>4–6</td>
<td></td>
</tr>
<tr>
<td>O-Methyl-L-triiodothyronine</td>
<td>10–40</td>
<td>1.5–3</td>
<td>2–3</td>
<td></td>
</tr>
<tr>
<td>3,5-Diiodo-DL-thyronine</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-Methyl diiodo-DL-thyronine</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL-Thyroxine</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-Methyl-DL-thyroxine</td>
<td>1</td>
<td>0</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Triiodothyroacetic acid</td>
<td>2</td>
<td>0.6</td>
<td>0.2*</td>
<td></td>
</tr>
<tr>
<td>O-Methyl triiodothyroacetic acid</td>
<td>2</td>
<td>0.5–0.7</td>
<td>0.14*</td>
<td></td>
</tr>
</tbody>
</table>

* The basal metabolic rate assays for triiodothyroacetic acid and for O-methyl triiodothyroacetic acid were done on thyroidectomized rats, with triiodothyronine as the reference standard. The activity is recorded assuming a value of 5 for triiodothyronine.

O-methyl triiodothyronine was in the range reported by Money et al. (23). O-Methyl triiodothyroacetic acid was about half as effective as triiodothyroacetic acid in suppression of 131I uptake by the thyroid gland (15), in goiter prevention and in enhancement of basal metabolic rate (16).

Effects on Mitochondrial Adenosinetriphosphatase

Lardy and Maley (24) found that thyroxine and several of its physiologically active analogues would stimulate the adeninetriphosphatase activity of rat liver mitochondria. The synthetic O-methyl ethers have been tested in this assay, and the results are summarized in Table III.

With intact mitochondria, thyroxine and triiodothyronine have relatively little ability to enhance ATPase compared with 2,4-dinitrophenol (24, 25). The methyl ethers of these two hormones were ineffective. On the other hand, methylation of triiodothyroacetic acid, which is one-third to one-half as effective as dinitrophenol, further increased the ability to stimulate ATPase. The response of mitochondrial ATPase to increasing concentrations of triiodothyroacetic acid and its O-methyl ether is depicted in Fig. 2. The maximal response to O-methyl triiodothyroacetic acid is greater than with triiodothyroacetic acid and is achieved at a lower concentration. Analogous curves were obtained for O-methyl tetraiodothyroacetic acid and tetraiodothyroacetic acid but the maximal responses were slightly less.

Triiodothyroethanol (22), which is closely related structurally to triiodothyroacetic acid and which is oxidized to that compound by rat kidney mitochondria, enhances ATPase. Methylation abolishes its activity.

Effects on Oxidative Phosphorylation

At 5 × 10^{-4} M or lower concentration, thyroxine and triiodothyronine uncouple oxidative phosphorylation only slightly in conventional experiments (26). With most substrates, triiodothyroacetic acid is somewhat more effective. The effects of the methyl ethers of thyroxine and triiodothyronine were not appreciably different from those of their respective parent compounds but the methyl ether of triiodothyroacetic acid was somewhat more effective than the acid (Table IV). This greater uncoupling activity was not abolished by 0.01 M fluoride.

The presence of sufficient malonate to block the oxidation of succinate produced from glutamate renders kidney mitochondria more susceptible to uncoupling by thyroxine (26). In repeated experiments with kidney mitochondria and malonate present, O-methyl thyroxine was less deleterious to phosphorylation than was thyroxine. Again triiodothyroacetic acid and its O-methyl ether were highly effective in uncoupling.

Metabolic Transformation

Mammalian mitochondria contain an enzyme system capable of degrading the alanine side chain of thyronine and iodinated...
thyronines to the corresponding substituted acetic acids (17,22). evaporated to dryness in a vacuum. The almost colorless residue
After the mixture had been kept in the cold overnight, it was
thyroxine (13) and triiodothyronine depresses goiter prevention
iodothyroacetic acid (622 mg, 1 mmole) was dissolved in absolute
methyl thyroxine in rat urine.
We have been unable to demonstrate the presence of O-methyl
thyroacetic acid in rat urine after the administration of the
respective P-labeled parent compounds. However, Roche, Michel, and de Gregorio (31) have recently reported finding O-
in the metabolism of thyroid hormones has not yet been answered.
substituted acetic acid formed from the corresponding unmethyl-
not convert O-methyl triiodothyronine to O-methyl iodothyro-
ated compounds.
In several carefully conducted and controlled experiments, we
have been unable to detect any conversion by rat kidney or liver
mitochondria of O-methyl thyroxine to either O-methyl thyro-
acetic acid or thyroacetic acid. Rat kidney mitochondria did not convert O-methyl triiodothyronine to O-methyl iodothyro-
acetic acid or triiodothyroacetic acid. The limit of detectability
in these experiments was approximately 1% of the amount of the
substituted acetic acid formed from the corresponding unmethyl-
ated compounds.

DISCUSSION
The question of whether O-methylation plays a significant role
in the metabolism of thyroid hormones has not yet been answered.
We have been unable to demonstrate the presence of O-methyl
thyroxine, O-methyl triiodothyronine, and O-methyl triiodo-
thyroacetic acid in rat urine after the administration of the respective P-labeled parent compounds. However, Roche, Michel, and de Gregorio (31) have recently reported finding O-
methyl thyroxine in rat urine.
Conceivably biological methylation might play a role in
determining relative activities of a given hormone in eliciting
different metabolic responses. For example, methylation of
thyroxine (13) and triiodothyronine depresses goiter prevention
much more than basal metabolic rate. In contrast, methylation
of triiodoacetic acid does not depress either of these activities.
We are continuing studies to determine whether biological
methylation of ortho iodo-substituted phenols is of importance
in the metabolism of thyroid hormone.

Syntheses
O-Methyl Triiodothyroacetic Acid. A. By Diazomethane—Tri-
iodothyroacetic acid (622 mg, 1 mmole) was dissolved in absolute
methanol (5 ml) and treated with an excess of ice-cold diazometh-
ane-ether solution. The reaction was almost instantaneous. After
the mixture had been kept in the cold overnight, it was
evaporated to dryness in a vacuum. The almost colorless residue
yielded 410 mg (63% of theoretical) of colorless needles from
dilute ethanol. The methyl ester of the O-methyl ether melted at
133-135°.

C14H20O3I5 (650.0)
Calculated: C 29.56, H 2.01, I 58.57
Found: C 29.73, H 1.92, I 58.85
The above methyl ester (162 mg, 0.5 mmole) was hydrolyzed
in 10 ml of 1 M NaOH in 50% ethanol at room temperature for 1
hour. Acidification with dilute HCl precipitated the free acid,
which was dried and recrystallized by dissolving in warm benzene
(10 ml) and adding petroleum ether (Skelly C) (30 ml). It was
dried over silica gel and P2O5 at 100° in a vacuum. When
heated slowly, the acid melted around 140°, then partly re-
solidified and finally cleared at 165-167°.4

C14H19O3I5 (636.0)
Calculated: C 28.32, H 1.74, I 59.86
Found: C 28.35, H 1.66, I 59.10

B. By Dimethyl Sulfate—Triiodothyroacetic acid (311 mg, 0.5
mmole) was dissolved in 50 ml of 1 M NaOH in 20% ethanol,
and while the solution was stirred at room temperature, dimethyl
sulfate (6 ml) was added in small increments. More alkali and
dimethyl sulfate were used, if necessary, until aliquots (2 to 3
drops) gave a negative phenol test with Folin-Dennis reagent.
The reaction mixture was acidified with dilute HCl, and the crude
product that precipitated was purified as in Procedure A.
The melting behavior was identical with that of the compound
prepared with diazomethane, and no depression of melting point
was observed when the two were mixed.

4 Dr. H. L. Saunders informed us that a preparation of this com-
ponent by Dr. J. Kerwin melted at 138-150°, resolidified, and re-
melted at 164-166°.

Table IV
Influence of thyroid hormones and their methyl ethers on oxidative phosphorylation
All data for a given substrate are averages of duplicates in a single experiment. The reaction mixture contained 2 mM ATP, 17 mM
potassium phosphate buffer, pH 7.4, 13 mM DL-β-hydroxybutyrate, 5 mM MgSO4, and rat kidney or liver mitochondria amounting to
1.4 to 1.8 mg of N per flask in the various experiments. Malonate, when present, was at 3.3 mM.

<table>
<thead>
<tr>
<th>Source of mitochondria</th>
<th>Additions</th>
<th>β-Hydroxybutyrate</th>
<th>Succinate</th>
<th>Glutamate</th>
<th>Glutamate + malonate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P:0</td>
<td>P:0</td>
<td>P:0</td>
<td>P:0</td>
</tr>
<tr>
<td>Rat kidney</td>
<td>None (control)</td>
<td>230</td>
<td>1.4</td>
<td>560</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Thyroxine</td>
<td>280</td>
<td>1.1</td>
<td>520</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>O-Methyl thyroxine</td>
<td>280</td>
<td>1.1</td>
<td>535</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Triiodothyronine</td>
<td>270</td>
<td>1.3</td>
<td>535</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>O-Methyl triiodothyronine</td>
<td>300</td>
<td>1.1</td>
<td>535</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Triiodothyroacetic acid</td>
<td>260</td>
<td>0.58</td>
<td>535</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>O-Methyl triiodothyroacetic acid</td>
<td>270</td>
<td>0.34</td>
<td>560</td>
<td>0.74</td>
</tr>
<tr>
<td>Rat liver</td>
<td>None (control)</td>
<td>370</td>
<td>2.3</td>
<td>500</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Thyroxine</td>
<td>310</td>
<td>2.4</td>
<td>430</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>O-Methyl thyroxine</td>
<td>370</td>
<td>2.1</td>
<td>460</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Triiodothyronine</td>
<td>310</td>
<td>2.4</td>
<td>410</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>O-Methyl triiodothyronine</td>
<td>350</td>
<td>2.1</td>
<td>420</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Triiodothyroacetic acid</td>
<td>280</td>
<td>2.0</td>
<td>390</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>O-Methyl triiodothyroacetic acid</td>
<td>290</td>
<td>1.4</td>
<td>380</td>
<td>1.3</td>
</tr>
</tbody>
</table>
O-Methyl tetraiodothyroacetic acid was prepared by Method B described above. The yield was 350 mg from 750 mg of tetraiodothyroacetic acid after several recrystallizations from benzene.

\[ \text{C}_{16}\text{H}_{11}\text{O}_{4}\text{I}_4 \] (761.9)

**Calculated:** C 25.64, H 1.32, I 66.66

**Found:** C 25.63, H 1.35, I 66.69

**Methyl Esters of Thyronines**

The esters were prepared by the method of Ashley and Harington (27) devised for the preparation of 3,5-diiodothyronine and thyroxine.

_**DL-Thyronine methyl ester**_ formed tiny, colorless needles from benzene or benzene-petroleum ether (Skelly B) that melted at 174–181° (decomposition).

_**3,5,3′-Triiodo-L-thyronine methyl ester**_ formed tiny, colorless crystals (from dilute ethanol) or cubes (from ethanol) that melted at 174–181° (decomposition).

_**O-Methyl Triiodo-L-thyronine**_ formed tiny, colorless needles from dilute ethanol; yield, 80 to 90% of the theoretical.

**Preparation of O-Methyl Thyronines with Diazomethane**

The method used was similar to that of Loeser, Ruland, and Trikojus (12), except that anisole was omitted from the reaction mixture. Methyl esters of thyronines (0.5 mmole) suspended in 10 ml of methanol were treated with diazomethane in ether (from dilute ethanol) or cubes (from ethanol) that melted at 174–181° (decomposition).

\[ \text{C}_{16}\text{H}_{11}\text{O}_{4}\text{I}_4 \] (665.0)

**Calculated:** C 28.89, H 2.12, N 2.10

**Found:** C 28.79, H 2.12, N 2.26

**O-Methyl-3,5-diiodothyronine**—yield, 180 mg from 400 mg of L-triiodothyronine.

\[ \text{C}_{16}\text{H}_{11}\text{O}_{4}\text{I}_4 \] (665.0)

**Calculated:** C 28.89, H 2.12, N 2.10

**Found:** C 28.79, H 2.12, N 2.26

**O-Methyl-3,5-diiodo-DL-thyronine**—Some difficulties were encountered with the purification of this compound. The solution of the crude product in ethanolic HCl was diluted with boiling water until it became turbid. The impurities were collected by repeated at 10-minute intervals until a 2-drop aliquot gave a negative Folin-Dennis phenol test. On acidification with dilute HCl and dilution with cold water, fine precipitates were obtained which formed tiny, colorless needles from dilute ethanol; yield, 80 to 90% of the theoretical.

**Calculated:** C 28.89, H 2.12, N 2.10

**Found:** C 28.79, H 2.12, N 2.26

**O-Methyl-N-benzy1-DL-thyronine**—Whereas the above two O-methyl ethers of iodothyrinones did not form good crystals from boiling 2 N HCl, O-methyl-DL-thyronine was readily purified from this solvent. Fine, colorless needles of the hydrochloride melted at 206–208° (decomposition).

**Calculated:** C 28.89, H 2.12, N 2.10

**Found:** C 28.79, H 2.12, N 2.26

The free O-methyl thyronine was crystallized in the manner described above; m.p., 210–212° (decomposition) when the temperature increment was 2° per minute. Clayton and Hems (29) reported that this compound, made by a different route, melted at 246–248° (decomposition).\(^4\)

**Methyl Esters of O,N-Dibenzoyl Thyronines**

The methyl esters of thyronines (1.5 mmoles), dissolved in dry pyridine (10 ml), were treated with benzoyl chloride (0.5 ml) at room temperature overnight. The crude product, which precipitated on addition of 5% NaHCO\(_3\) (5 ml) and cold water, was recrystallized from methanol or ethanol to form tiny, colorless needles. Yield, 80 to 90% of the theoretical.

**O,N-Dibenzy1-3,5-diiodothyronine Methyl Ester**—M.p., 137–139° (132–134° by Southwick et al. (28) and 133–134° by Harington and Pitt-Rivers (30)). The analytical data agreed with theory for \[ \text{C}_{16}\text{H}_{14}\text{O}_{4}\text{I}_4 \] (495.5).

**Calculated:** C 48.21, H 3.10, N 1.84, I 33.96

**Found:** C 48.15, H 3.12, N 2.03, I 33.86


**Calculated:** C 48.15, H 2.53, N 1.60, I 43.60

**Found:** C 48.30, H 2.26, N 1.47, I 43.52

**O-Methyl-N-benzy1-Thyronines**

The above methyl esters (1 mmole) were saponified with 0.5 N NaOH in 85% ethanol (44 ml) at room temperature for 30 minutes. With stirring, dimethyl sulfate (1 ml) was added to the mixture and heated for 1 hour with occasional shaking. The crystals slowly went into solution. Attempts to purify O-methyl thyronine methyl esters at this stage were unsuccessful. After saponification, the products were purified by dissolving in 0.33 N HCl in 85% ethanol and treatment with charcoal. They were crystallized by addition of 2 N sodium acetate to bring the pH to approximately 6.

**O-Methyl Triiodo-L-thyronine**—M.p., 212–214° (decomposition); yield, 180 mg from 400 mg of L-triiodothyronine.

**Calculated:** C 28.89, H 2.12, N 2.10

**Found:** C 28.79, H 2.12, N 2.26

**O-Methyl-3,5-diiodothyroacetic acid** after several recrystallizations from benzene. The esters were purified by dissolving in 0.33 N HCl in 85% ethanol and treatment with charcoal. They were crystallized in the manner described above; m.p., 210–212° (decomposition) when the temperature increment was 2° per minute. Clayton and Hems (29) reported that this compound, made by a different route, melted at 246–248° (decomposition).\(^4\)

**Calculated:** C 59.35, H 5.60, N 4.32, Cl 10.95

**Found:** C 59.07, H 5.93, N 4.58, Cl 10.89

The free O-methyl thyronine was crystallized in the manner described above; m.p., 210–212° (decomposition) when the temperature increment was 2° per minute. Clayton and Hems (29) reported that this compound, made by a different route, melted at 246–248° (decomposition).\(^4\)

**Calculated:** C 59.35, H 5.60, N 4.32, Cl 10.95

**Found:** C 59.07, H 5.93, N 4.58, Cl 10.89

\[^4\text{Dr. B. A. Hems (personal communication) found this compound to melt at 210–212°, depending greatly on the rate at which the sample is heated.}^\] Somewhat higher melting points were observed in a capillary tube as compared with a block.

**Calculated:** C 59.35, H 5.60, N 4.32, Cl 10.95

**Found:** C 59.07, H 5.93, N 4.58, Cl 10.89

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**Found:** C 59.07, H 5.93, N 4.58, Cl 10.89

\[^4\text{Dr. B. A. Hems (personal communication) found this compound to melt at 210–212°, depending greatly on the rate at which the sample is heated.}^\] Somewhat higher melting points were observed in a capillary tube as compared with a block.
**O-Methyl-N-benzoyl 3,5-diiodo-\(\alpha\)-thyronine**—M.p., 190–201°C.

\[ \text{C}_{13}\text{H}_{12}\text{O}_{3}\text{N}_{1} \text{I}_{2} \] (543.2)

**Calculated:** C 42.94, H 2.97, N 2.10, I 69.99

**Found:** C 43.22, H 3.19, N 1.69, I 39.10

**Summary**

1. The synthesis of the O-methyl ethers of 3,5-diiodothyronine, 3,3',5-triiodothyronine, 3,3',5-triiodothyroethanol, 3,3', 5-triiodothyroacetic acid and 3,3',5,5'-tetraiodothyroacetic acid is described.

2. O-Methyl triiodothyronine is as effective as triiodothyronine in stimulation of tadpole metamorphosis; it is less active than triiodothyronine in rat goiter prevention and in enhancing basal metabolic rate of rats. The activity of O-methyl triiodothyroacetic acid is approximately equal to that of triiodothyroacetic acid in each of these assays.

3. O-Methyl triiodothyroacetic acid enhances adenosinetriphosphatase activity of rat liver mitochondria to a greater extent than any other thyro-active substance tested. Within this series, it is also the most effective uncoupler of oxidative phosphorylation.

4. O-Methyl thyronine and O-methyl triiodothyronine are not converted to their corresponding thyroacetic acids by mitochondrial preparations which are capable of forming thyroacetic and triiodothyroacetic acids from their respective parent thyronines.

**Hydrolysis of O-Methyl-N-benzoyl Thyronines**

N-Benzoyl derivatives (1 mmole) were hydrolyzed by refluxing in acetic acid-concentrated HCl (20 ml each) for 4 hours. After evaporating to dryness in a vacuum (40° bath), the O-methyl thyronines produced were purified as for those prepared with diastemehane. Elementary analyses and melting points of the compounds prepared by these two routes were similar.

**O-Methyl Triiodothyroethanol**

Triiodothyroethanol (22) was methylated with diazomethane and recrystallized from dilute ethanol as tiny, colorless needles; m.p. 137-139°.

\[ \text{C}_{13}\text{H}_{10}\text{O}_{3}\text{NI}_{2} \] (543.2)

**Calculated:** C 28.94, H 2.28, I 69.99

**Found:** C 28.96, H 2.10, I 61.21

**REFERENCES**

Synthesis and Biological Activity of O-Methyl Derivatives of Thyroid Hormones

Kenkichi Tomita, Henry A. Lardy, Diane Johnson and Alan Kent