Thyromimetics IV

Synthesis of Some Derivatives of 3,3′,5-Triiodo-D and L-Thyronine

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Three pathways leading to the methyl ethers of 3,3′,5-triiodo-D and L-thyronine were explored. In these syntheses the acetyl, benzoyl, and trifluoroacetyl groups were studied as suitable amine-protecting groups, thus providing a number of hitherto unreported thyronine derivatives. Consideration of the over-all yields and the quality of the products from the various reaction sequences showed that the acetyl group was best suited for the protection of the amino group in these syntheses.

DURING A STUDY in which the thyromimetic activities of several ethers of thyromimetic substances were determined (1) it became desirable to prepare the methyl ethers of 3,3′,5-triiodo-D and L-thyronine (VII-D and L). The synthesis of the L-isomer has been described by Tomita *et al.* (2). The other reports dealing with VII-L contained biological data only (3, 4).

In the synthesis reported by Tomita *et al.* (2), 3,3′,5-triiodo-L-thyronine methyl ester (II*a*-L) was treated with diazomethane to give the methyl ether of II*a*-L. Basic hydrolysis followed by neutralization yielded VII-L in about 43% overall yield.

An alternate synthesis of VII-L using *N*-benzoyl-3,3′,5-triiodo-L-thyronine (IV*a*-L) and dimethyl sulfate was also explored by Tomita (2). However, difficulty in obtaining IV*a*-L caused the abandonment of this route. This route has since been used successfully in our laboratories.

DISCUSSION

Except for the work described by Tomita (2), the chemical preparation of derivatives of 3,3′,5-triiodo-D and L-thyronine (I-D and L) has not been described adequately in the literature. Consequently, two reaction sequences, in addition to the one using IV*a*, were studied in order to describe more fully such chemical work and to investigate alternate syntheses of VII-D and L. It was possible as a result of these studies to have available for biological and physical chemical examination a number of previously unavailable and unknown derivatives of I-D and L and methods for their preparation.

The syntheses varied since different acyl groups were used to protect the amine function in I during methylation of the 4′-phenolic group. Most of the reactions that were carried out are shown in Scheme I.

In the sequence leading to IV*a*, I was converted to its methyl or ethyl ester according to the method of Ashley and Harington (5). The methyl ester II*a* was purified and characterized as the hydrochloride, while the ethyl ester II*b* was isolated and purified as the free base.

The addition of benzoyl chloride to a suspension of either II*a* or II*b* in a mixture of tetrahydrofuran and 5% sodium bicarbonate resulted in the formation of the *N,O*-dibenzoyl-3,3′,5-triiodothyronine esters, III*a* and *b*. These esters were usually contaminated with a small amount of incompletely benzoylated material. This was removed conveniently either by triturating the crude reaction product with methanol or by extracting a chloroform solution of the reaction product with dilute base.

Basic hydrolysis of III*a* or *b* yielded IV*a* in what appeared to be a solvated form. Analyses of IV*a*-D and L have varied, despite numerous manipulations to prepare pure IV*a*-D and L. The elemental analyses have supported more consistently the structure indicated for IV*a* plus an additional mole of methanol than they have any other reasonable structure.

The structures of IV*a*-D and L have been substantiated by their conversion with methanolic hydrogen chloride to the esters V*a*-D and L, by appropriate infrared spectra, and by their conversion to *N*-benzoyl-3-[3,5-diiodo-4-(3-iodo-4-methoxyphenoxy)phenyl]-D and L-alanine methyl esters (VI*a*-D and L). V*a*-D and L and VI*a*-D and L have expected infrared spectra and give satisfactory elemental analyses. VI*a*-D and L prepared by complete methylation of IV*a*-D and L were identical to the products from the methylation of V*a*-D and L.

To demonstrate that IV*a*-D and L were indeed homogeneous, paper chromatograms were prepared in an isoamyl alcohol–*tert*-amyl alcohol–6 *N* ammonia system (1:1:2) and in an *n*-butanol–aqueous ammonia system (6). The components were detected by spraying with ninhydrin and Emerson's reagent (turns pink in the presence of *o*-iodophenols). The chromatograms showed IV*a*-D and L to consist of one major component contaminated with a trace amount of an unknown impurity. The major component was ninhydrin-negative and gave a positive reaction with Emerson's reagent.

Hydrolysis of VI*a* with a 1:1 mixture of acetic and concentrated hydrochloric acids for 4 hours, as reported by Tomita (2), or for 2 hours, as carried out subsequently by Blank, caused some cleavage of the ether. Evidence of this was obtained from paper chromatograms of the purified products using previously described systems and sprays (6). When the hydrolysis was performed for 18–24 hours with a 1:1 mixture of acetic acid and 6 *N* sulfuric acid, pure 3-[3,5-diiodo-4-(3-iodo-4-methoxyphenoxy)phenyl]-alanine (VII) was obtained.

In the route to VII, using *N*-acetyl-3,3′,5-triiodothyronine (IV*b*) as an intermediate, numerous

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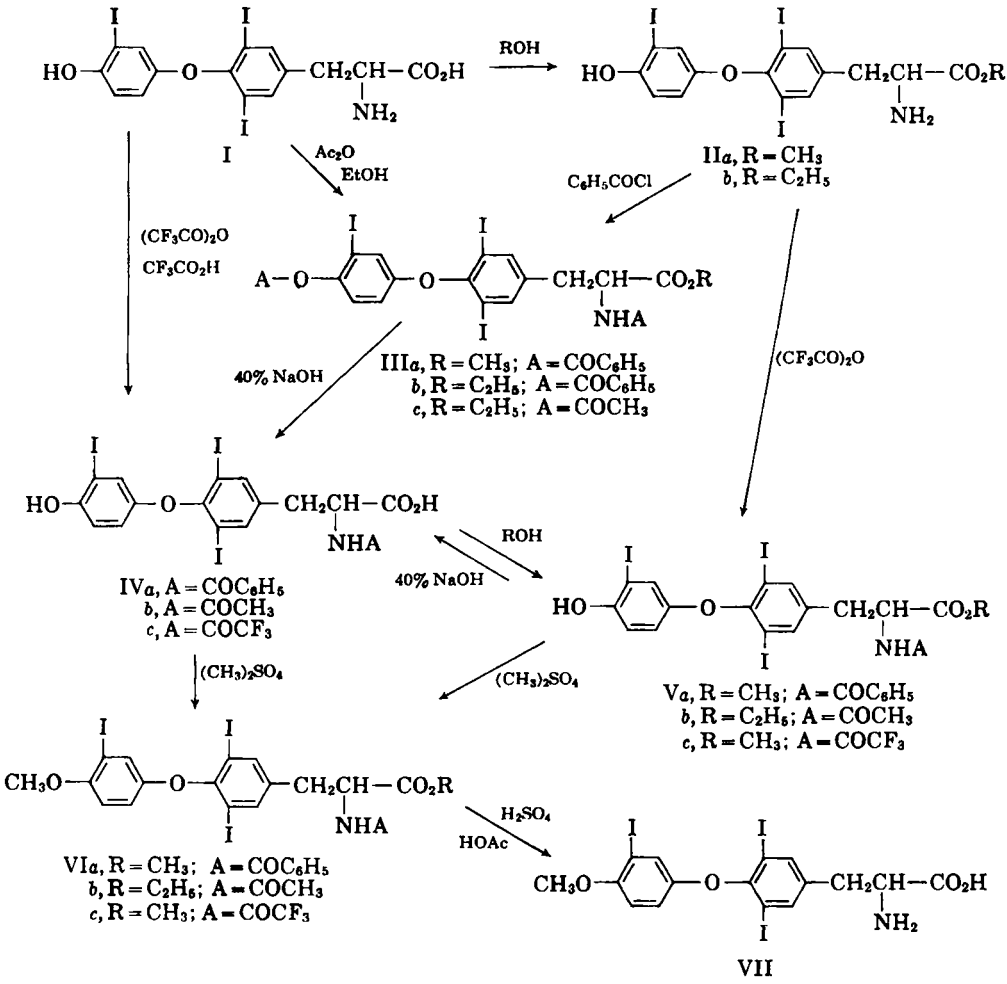


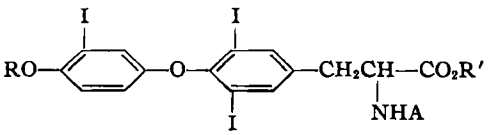
Scheme I

attempts were made to prepare IV*b* directly from I using conditions which were successful for the preparation of *N*-acetyl-3,5-diiodotyrosine (7) and *N*-acetylthyroxine (8). These attempts led to the recovery of unreacted impure I. The addition of ethanol to the reaction solution of I in acetic anhydride and aqueous sodium hydroxide, either during the acetylation or just prior to acidification of the acetylation solution, resulted in the isolation of an acetylated product.[1] That an *N*-acetyl derivative had been formed was shown by the facts that the isolated material was ninhydrin-negative, was acid insoluble, and had an amide peak in its infrared spectrum. In addition to the amide absorption at 6.09 μ, there were two additional carbonyl peaks in the infrared spectrum at 5.68 and 5.77μ which could correspond to a phenolic acetate and carboxylic acid ester, respectively. Moreover, the phenolic absorptions at 7.05–7.08 and 8.07–8.10 μ, which were present in IV*b* and V*b*, were not present in this material. Thus, this material was considered to be chiefly *N*,*O*-diacetyl-3,3',5-triiodothyronine ethyl ester (III*c*). Such a structure was in keeping with the report by Ashley and Harington (8) that *N*,*O*-diacetylthyroxine ethyl ester was formed under comparable conditions. Unfortunately, the isolation of pure III*c* was never accomplished, although several attempts were made. In one instance a sample was obtained which melted sharply and which showed only one spot on thin-layer and paper chromatography. Drying this material *in vacuo* for analysis caused the melting point to be lowered, and subsequent elemental analyses were unsatisfactory. Crude III*c*-D and L could, however, be converted with ethanolic hydrogen chloride to the readily purified and identified *N*-acetyl-3,3',5-triiodo-D and L-thyronine ethyl esters (V*b*-D and L).

Treatment of V*b* or the crude acetylation product with 40% sodium hydroxide yielded, after recrystallization from aqueous methanol, what seems to be a methanolate of *N*-acetyl-3,3',5-triiodothyronine (IV*b*) (on the basis of melting point and elemental analyses). This material behaved much like the *N*-benzoyl analog IV*a* since treatment of IV*b* with ethanolic hydrogen chloride gave V*b* comparable to the formation of V*a* from IV*a*. Then, too, both IV*a* and IV*b* were soluble in dilute aqueous sodium carbonate, both were ninhydrin-negative, and on paper chromatograms both showed a single spot which was positive to Emerson's reagent and negative to ninhydrin.

[1] The need for alcohol in such acetylations has been reported previously for the preparation of *N*-acetylthyroxine (9).

TABLE I.—DERIVATIVES OF 3,3′,5-TRIIODO-D AND L-THYRONINE



No.	R	R′	A	M.p., °C.	Recrystal. Solvent	Optical Rotation in degrees $[\alpha]_D^{25}$[a]	Formula	Anal., % Calcd. C	Calcd. H	Calcd. I	Found C	Found H	Found I
IIa-D	H	CH_3	H	213–215[b]	CH_3OH-$(C_2H_5)_2O$	−9.4[c]	$C_{16}H_{15}ClNO_4$[d]	27.40	2.16	54.27	27.77	2.30	54.01
a-L	H	CH_3	H	215[b]	CH_3OH-$(C_2H_5)_2O$	+8.9[c]					27.66	1.97	54.49
b-D	H	C_2H_5	H	149–150	C_2H_5OH-H_2O	−28.2[e]	$C_{17}H_{16}I_2NO_4$	30.07	2.38	56.07	30.32	2.25	56.14
b-L	H	C_2H_5	H	148–150	C_2H_5OH-H_2O	+27.7[e]					30.44	2.51	55.62
IIIa-D	C_6H_5CO	CH_3	COC_6H_5	182–183	EtOAc-pet. ether	+34.4[f]	$C_{30}H_{22}I_2NO_6$	41.26	2.54	43.60	41.54	2.48	43.56
a-L	C_6H_5CO	CH_3	COC_6H_5	184–185	CH_3OH	−37.8[f]	[g]				41.20	2.47	43.89
b-D	C_6H_5CO	C_2H_5	COC_6H_5	165–167	EtOAc-pet. ether	+28.6[f] (c,1.2)	$C_{31}H_{24}I_2NO_6$	41.96	2.73	42.91	42.33	2.78	43.16
b-L	C_6H_5CO	C_2H_5	COC_6H_5	169–171	EtOAc-pet. ether	−28.1[f]					42.08	2.72	42.84
IVa-D	H	H	COC_6H_5	148	CH_3OH-H_2O	+15.6[e]	$C_{23}H_{20}I_2NO_6$[h]	35.09	2.56	48.37	35.46	2.60	49.61
a-L	H	H	COC_6H_5	148	CH_3OH-H_2O	−14.8[e]					35.34	2.49	48.43
b-D	H	H	$COCH_3$	140	CH_3OH-H_2O	−16.6[i]	$C_{18}H_{16}I_2NO_6$[h]	29.81	2.52	52.51	29.86	2.51	52.95
b-L	H	H	$COCH_3$	136–138	CH_3OH-H_2O	+14.1[i]					29.78	2.23	52.94
c-D	H	H	$COCF_3$	199–201	EtOAc-pet. ether	−4.6[f]	$C_{17}H_{11}F_3I_2NO_5$	27.33	1.48	50.97	27.58	1.57	51.18
c-L	H	H	$COCF_3$	197–199	EtOAc-pet. ether	+4.4[f]					27.57	1.68	51.19
Va-D	H	CH_3	COC_6H_5	112–114	CH_3OH-H_2O	+39.8[e]	$C_{23}H_{18}I_2NO_5$	35.92	2.36	49.50	36.24	2.46	49.37
a-L	H	CH_3	COC_6H_5	113–115	CH_3OH-H_2O	−33.6[e]					36.12	2.41	49.60
b-D	H	C_2H_5	$COCH_3$	150–152	C_2H_5OH-H_2O	−9.5[i]	$C_{19}H_{18}I_2NO_5$	31.65	2.52	52.80	31.87	2.45	52.85
b-L	H	C_2H_5	$COCH_3$	147–148	50% Aq.C_2H_5OH	+8.0[i]					31.69	2.43	52.59
c-D	H	CH_3	$COCF_3$	137–139	CH_3OH-H_2O	+5.9[e] (c,0.9)	$C_{18}H_{12}F_3I_2NO_5$	28.41	1.72	50.03	28.57	1.85	50.01
c-L	H	CH_3	$COCF_3$	139–141	CH_3OH-H_2O	−6.5[e] (c,0.9)					28.52	1.77	50.25
VIa-D	CH_3	CH_3	COC_6H_5	100–103	C_2H_5OH-H_2O	+44.2[i] (c,0.8)	$C_{24}H_{20}I_2NO_5$	36.81	2.57	48.61	36.96	2.48	48.54
a-L	CH_3	CH_3	COC_6H_5	102–105	C_2H_5OH-H_2O	−45.8[i]					37.00	2.69	48.55
b-D	CH_3	C_2H_5	$COCH_3$	125–128	EtOAc-pet. ether	−9.3[i]	$C_{20}H_{20}I_2NO_5$	32.68	2.74	51.79	32.61	2.82	51.85
b-L	CH_3	C_2H_5	$COCH_3$	127–129	EtOAc-pet. ether	+8.8[i]					32.52	2.79	51.33
c-D	CH_3	CH_3	$COCF_3$	120–122	70% Aq.C_2H_5OH	−8.6[i]	$C_{19}H_{15}F_3I_2NO_5$	29.44	1.95	49.12	29.70	2.04	49.08
c-L	CH_3	CH_3	$COCF_3$	115–117	70% Aq.C_2H_5OH	+7.3[i]					29.60	2.02	48.98
VII-D	CH_3	H	H	234–236[b]	C_2H_5OH-H_2O[j]	−21.6[e] (c,1.1)	$C_{16}H_{14}I_2NO_4$		[k]				
-L	CH_3	H	H	229–230[b]	C_2H_5OH-H_2O[j]	+18.0[e]	$C_{16}H_{16}I_2NO_5$[l]		[k]				
VIII-L	CH_3	C_2H_5	H	145[b]	$(CH_3)_2CO$	+17.2[i]	$C_{18}H_{19}ClI_2NO_4$[d]	29.63	2.63	52.19	29.78	2.65	51.94

[a] c = 1 unless indicated. [b] With decomposition. [c] Determined in methanol. [d] Hydrochloride. [e] Determined in ethanol–1 *N* hydrochloric acid (3:1 by volume). [f] Determined in ethyl acetate. [g] Also reported in *Reference 2*. [h] Methanolate. (See *text*.) [i] Determined in ethanol. [j] Recrystallized as described under *Experimental*. [k] See *Reference 1*. [l] Hydrate.

Unfortunately, NMR studies of IV*b*-L in deuteroacetone showed no evidence of methanol. No explanation can be presented currently to explain these anomalous data.

Methylation of V*b* to VI*b* and hydrolysis of VI*b* with acetic and sulfuric acids to VII-D and L proceeded in a straightforward manner.

In an effort to circumvent the *O*-acylation which occurred when I was acetylated or benzoylated and which necessitated at least an additional hydrolysis step, the use of the *N*-trifluoroacetyl group was investigated. The preparation of *N*-trifluoroacetyl-3,3',5-triiodo-D and L-thyronines (IV*c*-D and L) was accomplished conveniently in good yield by adding trifluoroacetic acid and anhydride to I. The methyl ester methyl ether VI*c* was obtained with difficulty by treating IV*c* with dimethyl sulfate. This conversion required two treatments with dimethyl sulfate since the first treatment gave mainly the impure methyl ester V*c*. Further treatment of impure V*c* with dimethyl sulfate gave VI*c*.

Pure V*c* was prepared by stirring IV*c* with methanolic hydrogen chloride or by adding trifluoroacetic anhydride to II*a* hydrochloride. Hydrolysis of analytically pure samples of VI*c*-D and L with 10% sodium hydroxide yielded impure VII-D and L. These samples of VII were grayish-purple in color, had lower melting points, lower optical rotations, and poorer paper chromatograms than VII made using VI*b*. Re-examination of VI*c*-D and L by paper chromatography showed these materials to contain significant amounts of unetherified material. Since the intermediates IV*c* and V*c* can withstand only limited exposure to base without loss of the *N*-trifluoroacetyl group it was apparent from these results that the etherification did not go to completion under the conditions of these experiments.

A comparison of the over-all percentage yields of VII and the purity of the VII from the three routes showed that the pathway using IV*b* was best—45% *versus* 15% *via* IV*a* *versus* 30% *via* IV*c*. Thus, the best route described here compares quite favorably with that described by Tomita (2), particularly if it is felt that the preparation and use of diazomethane is a problem.

To determine whether over-all retention of configuration occurred in these syntheses, VII-L (prepared *via* IV*b*) was hydrolyzed with a mixture of hydriodic and acetic acids to give I-L with $[\alpha]_D^{25} = +25.5°$, c = 1 in ethanol − 1 *N* hydrochloric acid (3:1 by volume). The starting material had $[\alpha]_D^{25} = +22.6°$, c = 2 in the above solvent system at a dilution of 4:1.

The ethyl ester of VII-L (VIII-L) was prepared simply by stirring VII-L with ethanolic hydrogen chloride.

Table I lists the compounds prepared, appropriate physical constants, and analytical data.

EXPERIMENTAL[2]

3,3',5-Triiodo-D and L-Thyronine Methyl Esters (IIa-D and L).—II*a*-D and L hydrochlorides were prepared according to the method of Ashley and Harington (5). The hydrochlorides were dissolved in aqueous methanol and neutralized with 5% sodium carbonate. The precipitated II*a* was cooled, filtered, washed with water, and recrystallized from tetrahydrofuran-water to give 80–85% of product, m.p. 195–197°.[3]

3,3',5-Triiodo-D and L-Triiodothyronine Ethyl Esters (IIb-D and L).—These esters were prepared in 45–55% yield using the method employed for II*a* with ethanol instead of methanol.

N,O-Dibenzoyl-3,3',5-Triiodo-D and L-Thyronine Methyl and Ethyl Esters (IIIa-D and L and IIIb-D and L).—A solution of 2.5 mmoles of II*a* or II*b* in 50 ml. of tetrahydrofuran was treated with 12 ml. of 5% sodium bicarbonate, 1 Gm. of solid sodium bicarbonate, and 1 ml. of benzoyl chloride, and stirred at room temperature for 2 hours. The mixture was poured into 3–4 vol. of ice-water. The resulting gum was dissolved in chloroform, and the chloroform solution was washed three times with 10% sodium hydroxide, three times with water, and dried over sodium sulfate. The dried solution was concentrated, and the syrupy residue was triturated with aqueous methanol or petroleum ether to produce impure solid II*a* or *b*. After recrystallization, the yields were 45–65%.

Acidification of the aqueous alkaline washes from above gave a small amount of impure IV*a*.

N-Benzoyl-3,3',5-triiodo-D and L-Thyronines (IVa-D and L).—A solution of 1 mmole of III*a* or *b* in 25 ml. of methanol and 5 ml. of 40% sodium hydroxide was stirred 1 hour at room temperature, diluted with water, cooled, and acidified. The precipitate was filtered, washed with water and ether, and recrystallized. Yield 75–90%.

N-Benzoyl-3,3',5-triiodo-D and L-Thyronine Methyl Esters (Va-D and L).—These were prepared in 55–80% yield using the method described for V*b*.

N-Benzoyl-3-[3,5-diiodo-4-(3-iodo-4-methoxyphenoxy)phenyl]-D and L-Alanine Methyl Esters (VIa-D and L).—These compounds could be prepared from either IV*a* or V*a* under similar conditions. However, the product from V*a* was purer and more easily purified.

To a solution of 1 mmole of V*a* in 25 ml. of 5% sodium carbonate and 25 ml. of tetrahydrofuran was added 1.3 ml. of dimethyl sulfate. The mixture was stirred at room temperature for 2.5 hours. The pH of the mixture was checked periodically to insure the basicity of the reaction mixture. More base was added if needed. The mixture was diluted with cold water to precipitate a gum. The gum was dissolved in chloroform; the chloroform was washed once with base and several times with water. The chloroform was dried over sodium sulfate and evaporated to produce an oil. Trituration of the oil with petroleum ether gave a solid which was filtered, washed with petroleum ether, and recrystallized. Yield, 55–70%.

Acetylation of 3,3',5-Triiodo-D and L-Thyronines (IIIc?).—To a cooled stirred solution of 3.25 Gm. (5 mmoles) of I in 100 ml. of 2 *N* sodium hydroxide was added slowly 1.5 ml. of acetic anhydride. Stirring was continued for 2 hours at room temperature when 50 ml. of ethanol was added. The solution was diluted with water, cooled, and acidified with dilute hydrochloric acid. The white precipitate was filtered, washed with water, and dried to give 3.4 Gm. of material with an indefinite melting point.

[2] All melting points were taken in a Thomas-Hoover capillary melting point apparatus and are corrected. Microanalyses were performed by Mrs. Doris Rolston and coworkers, and optical rotations were determined by Mr. J. Walter Hamill and staff, Analytical and Physical Chemistry Section, Smith Kline and French Laboratories, Philadelphia, Pa.

[3] The DL-isomer has been reported to melt at 178.5–179° dec. (10) and the L-isomer at 174–181° dec. (2).

Recrystallization of 2 Gm. of this material from boiling toluene gave 1.2 Gm. of a product, melting about 140° after washing with petroleum ether. A small sample (300 mg.) was then suspended in 40 ml. of ether and stirred for 30 minutes. Most of the solid dissolved. The insoluble portion was filtered, and the filtrate was added to 100 ml. of petroleum ether to precipitate 200 mg. of solid, m.p. 189°. This material showed one spot on thin-layer (silica gel plates developed with methanol) and paper chromatograms (ethanol–water–mineral oil, 2:1:10). After drying at 80° *in vacuo*, the material melted at 179–181° and gave unsatisfactory elemental analyses.

N-Acetyl-3,3′,5-Triiodo-D and L-Thyronines (IVb-D and L).—*Preparation A.*—A solution of 3.4 Gm. of crude acetylation product in 100 ml. of ethanol and 20 ml. of 40% sodium hydroxide was stirred for 1 hour at room temperature. The solution was diluted with water and acidified with dilute hydrochloric acid. The resulting solid was filtered, washed with water, and dried to give 3 Gm. of crude product.

Preparation B.—A solution of 2.5 mmoles of V*b* in 50 ml. of ethanol and 10 ml. of 40% sodium hydroxide was stirred 1 hour at room temperature, and the product was isolated as described under *Preparation A*. Yield, 84–98%.

N-Acetyl-3,3′,5-triiodo-D and L-Thyronine Ethyl Esters (Vb-D and L).—*Preparation A.*—A suspension of 4.2 Gm. of crude acetylation material in 50 ml. of absolute ethanol was cooled and saturated with dry hydrogen chloride. The resulting solution was evaporated, and the solid residue was evaporated twice again with fresh portions of ethanol. The residue was triturated with water, filtered, washed with water, and recrystallized.

Preparation B.—A suspension of 0.8 mmole of IV*b* in 35 ml. of absolute ethanol was saturated with dry hydrogen chloride and was isolated and purified as indicated in *Preparation A*. Yield, 67–93%.

N - Acetyl - 3 - [3,5 - diiodo - 4 - (3 - iodo - 4 - methoxyphenoxy)phenyl]-D and L-Alanine Ethyl Esters (VIb-D and L).—A solution of 4.2 mmoles of V*b* in 50 ml. of tetrahydrofuran and 50 ml. of 5% sodium carbonate was treated with 3.5 ml. of dimethyl sulfate. The reaction was performed, and the crude product was isolated as described in the preparation of VI*a*. The crude gummy product was solidified by stirring, cooling, and scratching with water. Yield, 65–70%.

On some occasions the gum was dissolved in ethyl acetate, and the organic solution was washed with water, dried (Na_2SO_4), and distilled. The residue was triturated with petroleum ether to produce solid.

N-Trifluoroacetyl-3,3′,5-triiodo-D and L-Thyronines (IVc-D and L).—To a cooled stirred suspension of 5 mmoles of I in 45 ml. of ethyl acetate was added 9 ml. of trifluoroacetic acid and 1.5 ml. of trifluoroacetic anhydride. Stirring and cooling were continued for 1 hour. The solution was washed with water, once with 5% sodium bicarbonate, and twice with saturated saline solution. The combined aqueous phases were extracted twice with ethyl acetate. The combined ethyl acetate layers were washed once with water and dried over sodium sulfate. Removal of the ethyl acetate left a solid which was dissolved in ether and reprecipitated with petroleum ether. This solid melted at 180–185° and represented a 91% yield of product.

N-Trifluoroacetyl-3,3′,5-triiodo-D and L-Thyronine Methyl Esters (Vc-D and L).—*Preparation A.*—The esters were prepared in 60–65% yield using IV*c* and methanolic hydrogen chloride in a manner analogous to that described for the preparation of V*b*.

Preparation B.—To a suspension of 1 mmole of II*a* hydrochloride in 5 ml. of ethyl acetate and 5 ml. of chloroform was added with stirring 0.5 ml. of trifluoroacetic anhydride. Stirring was continued for an additional 10 minutes, and the resulting clear solution was diluted with an equal volume of ethyl acetate. The solution was washed once with 5% sodium bicarbonate and twice with water. The combined aqueous phases were washed twice with ethyl acetate, and the combined organic phases were dried (Na_2SO_4). The solid residue obtained upon removal of the solvents melted at 147° after recrystallization.[4] Yield, 600 mg. (79%).

N - Trifluoroacetyl - 3 - [3,5 - diiodo - 4 - (3-iodo-4-methoxyphenoxy)phenyl]-D and L-Alanine Methyl Esters (VIc-D and L).—To a stirred suspension of 2.5 mmoles of IV*c* and 3 Gm. of sodium carbonate in 50 ml. of tetrahydrofuran was added 1.5 ml. of dimethyl sulfate. The product was isolated as were VI*a* and *b*. In this way an impure product was obtained which appeared to be predominantly V*c*.

Further treatment of this material in tetrahydrofuran with 1 ml. of dimethyl sulfate and 2 Gm. of sodium carbonate in 5 ml. of water produced the desired methyl ester methyl ether in 40–45% yield.

Treatment of V*c* with dimethyl sulfate under these conditions gave only unreacted starting material.

3 - [3,5 - Diiodo - 4 - (3 - iodo - 4 - methoxyphenoxy)phenyl]-D and L-Alanines (VII-D and L).—*Preparation A.*—A mixture of 1 mmole of VI*a* or *b* in 35 ml. of acetic acid and 35 ml. of 6 *N* sulfuric acid was refluxed for 18–24 hours. The resulting solution was diluted with water and cooled to give a precipitate of what was probably the sulfate of VII. This was not isolated; the suspension was adjusted to pH 5–6 with 10% sodium hydroxide. This precipitate was filtered, washed with water, and purified by several isoelectric precipitations from hot ethanolic hydrochloric acid by the addition of hot water and hot 2 *N* sodium acetate. Yield, 75–90%.

Preparation B.—A solution of 500 mg. (0.645 mmole) of VI*c* in 25 ml. of methanol and 2.5 ml. of 10% sodium hydroxide was allowed to stand at room temperature overnight. The solution was diluted with water, cooled, and adjusted to pH 4–5 with acetic acid. The product was isolated and purified as above. Yield, 82%.

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