## 541. *The Synthesis of Thyroxine and Related Substances. Part XIV.*[1] *The Preparation and Chromatography of Some Iodinated Thyronines.*

By J. S. Varcoe and W. K. Warburton.

3-Iodo-L-thyronine has been prepared from 3,5-di-iodo-L-thyronine, and converted into 3,3′-di- and 3,3′,5′-tri-iodo-L-thyronine. 3′-Iodo- and 3′,5′-di-iodo-L-thyronine have been prepared from L-thyronine. The purity of these compounds has been established by a quantitative chromatographic method, which is described.

It is well known that thyroid extracts depress the level of circulating cholesterol in hypothyroid and euthyroid humans.[2] but the routine administration of thyroid hormones for this purpose may be undesirable, and we have prepared some derivatives of L-thyronine that contain less than two iodine atoms in the phenylalanine ring (ring A), in the hope that these might lower serum cholesterol but have a relatively small effect on other metabolic processes.

It has been found in these Laboratories that there are often important differences in biological action between D- and L-derivatives of thyronine. This paper reports the preparation of relatively large quantities of some derivatives of L-thyronine containing only small and accurately known amounts of related compounds. All the required

[1] Part XIII, *J.*, 1953, 1448.
[2] Lévy and Lévy, *Bull. Acad. méd.* (*Paris*), 1931, **105**, 666; Gildea, Man, and Peters, *J. Clin. Invest.*, 1939, **18**, 739; Turner and Steiner, *ibid.*, 1939, **18**, 45; Barnes, *Lancet*, 1959, II, 149.

compounds have been prepared in optically inactive forms by earlier workers, but the L-forms have never been described.

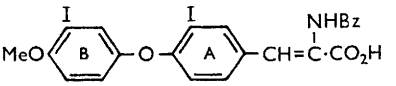
Roche, Michel, and Wolf [3] prepared 3-iodo-DL-thyronine from 3,5-di-iodo-DL-thyronine by partial catalytic reduction. The yield of pure 3-iodothyronine was not reported. We have prepared 3-iodo-L-thyronine by substantially the same method as the French workers, but the hydrochloride of 3,5-di-iodo-L-thyronine is too soluble in hot 2N-hydrochloric acid to be separated by the method reported for the DL-series.

In order to follow the course of the partial reduction, and of later iodinations, we have developed a method of paper chromatography that permits as little as 1% of thyronine or 3,5-di-iodothyronine in 3-iodothyronine to be estimated, and the same method has been used to check the purity of other iodinated thyronines.

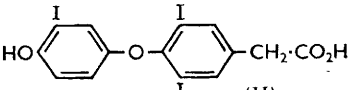
The hydrochloride of 3-iodo-L-thyronine crystallizes well from a mixture with L-thyronine in hydrochloric acid, provided that the concentration of the latter amino-acid does not exceed about 30%. However, if the 3-iodo-L-thyronine contains more than about 5% of 3,5-di-iodo-L-thyronine, some of the hydrochloride of the latter compound crystallizes with that of 3-iodo-L-thyronine and cannot be reduced to less than 1% except with great loss. It is therefore necessary to check the composition of the crude reduction products obtained by using varying amounts of hydrogen.

3,3',5'-Tri-iodo-L-thyronine was prepared in about 70% yield by iodination of 3,3'-di-iodo-L-thyronine in aqueous diethylamine with 120% of the calculated amount of iodine. However, iodination of 3-iodo-L-thyronine, by Roche, Michel, and Wolf's method,[3] gave a product containing about 5% of 3,3',5'-tri-iodo-L-thyronine and about 10% of 3-iodo-L-thyronine. We finally obtained 3,3'-di-iodo-L-thyronine containing less than 1% each of 3-iodo- and 3,3',5'-tri-iodo-L-thyronine by treating 3-iodo-L-thyronine with 140% of the calculated amount of iodine and separating the di- from the tri-iodothyronine as their ammonium salts in 1,1-dimethylpropan-1-ol on a cellulose-powder column. Both these compounds have been reported to occur in Nature.[4,5]

The ease with which 3,3'-di-iodothyronine loses iodine from ring B deserves comment. Reduction and demethylation of α-benzamido-3-iodo-4-(3-iodo-4-methoxyphenoxy)-cinnamic acid (I) with hydriodic or hydrobromic acid, even in the presence of red phosphorus,[6] gave only 3-iodo-DL-thyronine. We have found that boiling 3,3'-di- or 3,3',5'-tri-iodo-L-thyronine with 2N-hydrochloric acid for 30 min. causes less than 1% loss of iodine. However, when a solution of 3,3'-di-iodo-L-thyronine in 0·5N-alcoholic hydrochloric acid was left for 48 hours at room temperature, $[\alpha]_D^{20}$ changed from +18·8° to +20·4°, which suggests that there had been some conversion into 3-iodo-L-thyronine. On the other hand, there was no noticeable decomposition under the alkaline conditions used for chromatography during short periods.




(I)




(II)

The loss of iodine from ring B of thyroxine-like compounds is not confined to 3,3'-di-iodothyronine. Meltzer, Lustgarten, and Fischmann [7] attempted to demethylate methyl 3,5-di-iodo-4-(3-iodo-4-methoxyphenoxy)phenylacetate under acid conditions, but were unable to avoid loss of iodine. Similarly, 3,5-di-iodo-4-(4-hydroxy-3-iodophenoxy)-phenylacetic acid (II) loses iodine when boiled with hydriodic acid, but we have not been

[3] Roche, Michel, and Wolf, *Bull. Soc. chim. France*, 1957, 462.
[4] Roche, Michel, Wolf, and Nunez, *Biochim. Biophys. Acta*, 1956, **19**, 308.
[5] Roche, Michel, and Nunez, *Bull. Soc. Chim. biol.*, 1958, **40**, 361.
[6] Gemmill, Anderson, and Burger, *J. Amer. Chem. Soc.*, 1956, **78**, 2434.
[7] Meltzer, Lustgarten, and Fischmann, *J. Org. Chem.*, 1955, **22**, 1577.

able to isolate the pure di-iodo-compound from the reaction mixture. Finally, 3',5'-di-iodothyronine readily loses iodine when warmed in the dry state or in neutral solution, and demethylation of its ether requires mild conditions.[8]

We have iodinated L-thyronine by a modification of the method of Clayton and Hems [9] and have prepared pure 3',5'-di-iodo-L-thyronine without isolating 3'-iodo-L-thyronine. The hydrochloride of the di-iodo-compound is very sparingly soluble in boiling 2N-hydrochloric acid, and traces of 3'-iodo-L-thyronine are easily removed from 3',5'-di-iodo-L-thyronine by treatment with hydrochloric acid. 3'-Iodo-L-thyronine was conveniently prepared by treating thyronine with an excess of iodine and removing the di-iodothyronine as its hydrochloride.

Paper chromatography in 1,1-dimethylpropan-1-ol and 6N-ammonia separates any mixture of thyronine and iodinated thyronines likely to be found in preparative work, and it can be used to examine up to $10^{-4}$ g. of product. Systems containing even freshly purified dioxan lead to artefacts through elimination of iodine. This property of dioxan has been observed before,[10] and it is probably responsible for the large number of spots observed by Donhoffer *et al.*[11] when they chromatographed several thyroxine-like compounds in solvents containing dioxan.

The spots may be detected through the phenolic hydroxyl group by spraying with a mixture of potassium ferricyanide and ferric chloride (Barton's reagent).[12] This gives bright spots of Turnbull's blue, which are well graded in size and intensity according to the concentration of the amino-acid. A more sensitive method of detection, depending on the catalysis by iodide ion of the reduction of ceric salts by arsenious acid,[13] is less suitable for comparative work. Barton's reagent will clearly detect as little as $5 \times 10^{-7}$ g. of thyronine and $10^{-6}$ g. of any of the iodinated thyronines on a chromatogram; and the addition of, *e.g.*, $10^{-6}$ g. of thyronine to $10^{-4}$ g. of pure 3-iodothyronine, followed by chromatographic separation, gives a spot for thyronine similar in size and intensity of colour to a reference spot containing only $10^{-6}$ g. of thyronine. The chromatograms are stable for at least two years.

For refined work it is best to dry the chromatogram and run it again in the same solvent and in the same direction [14] before spraying. This increases the separation and improves the definition; to estimate as little as 1% of 3,5-di-iodo- in 3-iodo-thyronine it is in fact essential to run the paper twice.

Biological tests carried out in these Laboratories have shown that none of these compounds causes sufficient depression of blood cholesterol in rats to encourage further testing.[15] Oxygen-consumption tests in both rats and mice, and goitre-prevention tests in rats, showed no significant activity.[16]

## Experimental

M. p.s are corrected. Rotations refer to 1 : 1 (v/v) N-HCl–EtOH solutions (*c* 1).

*Paper Chromatography.*—The compounds to be analysed were dissolved in absolute ethanol–2N-ammonia (3 : 1 v/v; 25 mg. in 5 ml.) and applied to Whatman No. 1 chromatography paper (10″ × 10″) within 1 hr. with an " Agla " micrometer syringe. The spots (100 μg.) were applied in 2 μl. portions, which were dried at room temperature with a hair-drier. Smaller quantities of reference spots were applied similarly, from solutions diluted 1 in 10. Ascending chromatograms were equilibrated for ½ hr. and run for about 18 hr. at 25° in redistilled 1,1-dimethylpropan-1-ol saturated with 6N-ammonia. After drying at 37° or less, the papers were sprayed

[8] Block and Powell, *J. Amer. Chem. Soc.*, 1942, **64**, 1070.
[9] Clayton and Hems, *J.*, 1950, 840.
[10] Maclagan, Bowden, and Wilkinson, *Biochem. J.*, 1957, **67**, 5.
[11] Donhoffer, Jarai, Mestyán, Szegvári, and Varnai, *Naturwiss.*, 1958, **45**, 264.
[12] Barton, Evans, and Gardner, *Nature*, 1952, **170**, 249.
[13] Bowden, Maclagan, and Wilkinson, *Biochem. J.*, 1955, **59**, 93.
[14] Jeanes, Wise, and Dimler, *Analyt. Chem.*, 1951, **23**, 415.
[15] Cuthbertson, Elcoate, Ireland, and Mills, unpublished work.
[16] Tomich, Woollett, and Pratt, *J. Endocrinol.*, 1960, **20**, 65.

with about 10 ml. of an aqueous solution made by mixing equal volumes of 1% ferric chloride and 1% potassium ferricyanide solution just before use. They were immediately washed for 2 min. with 2N-hydrochloric acid, then twice with water, blotted, and dried at room temperature.

The $R_F$ values of some amino-acids in this system at 25° are given in the annexed Table.

| Thyronine | $R_F$ | Thyronine | $R_F$ |
|---|---|---|---|
| Unsubst. | 0·26 | 3′,5′-Di-iodo- | 0·15 |
| 3-Iodo- | 0·35 | 3,5,3′-Tri-iodo- | 0·33 |
| 3′-Iodo- | 0·22 | 3,3′,5′-Tri-iodo- | 0·19 |
| 3,3′-Di-iodo- | 0·22 | Tetra-iodo- | 0·22 |
| 3,5-Di-iodo- | 0·42 | | |

3-*Iodo*-L-*thyronine*.—The amount of hydrogen to be used with each batch of Raney nickel, made by the method of Covert and Adkins,[17] was found by carrying out hydrogenations as described in the next paragraph, using, in the first trial, 100, 150, 200, and 250 ml. of hydrogen. The crude, dry product was then dissolved in hot 2N-hydrochloric acid (150 ml.) and taken to pH 5·5 with concentrated ammonia. The total solid was collected after refrigeration and washed with water, and its composition found by chromatography. Products containing less than 3% of 3,5-di-iodo-L-thyronine and less than 30% of L-thyronine were recrystallized as the hydrochlorides, and the weights and compositions of these were determined.

3,5-Di-iodo-L-thyronine (5·25 g.) was dissolved in 95% ethanol (200 ml.), and concentrated aqueous ammonia (50 ml.), and Raney nickel (5·0 ml.) were added. The mixture was hydrogenated during about 30 min. at atmospheric temperature and pressure, and after filtration the solvent was removed under reduced pressure. The residue was dissolved in boiling 2N-hydrochloric acid (200 ml.), treated with a little sodium hydrogen sulphite and charcoal, filtered, and allowed to cool slowly to room temperature. The colourless needles were collected next day and recrystallised from 2N-hydrochloric acid (35 ml./g.) by allowing the solution to cool slowly and remain at room temperature for 5 hr. When pure, the hydrochloride was dissolved in hot 0·5N-hydrochloric acid (40 ml./g.) and the pH slowly adjusted to 5·5 at the b. p. with concentrated ammonia. Needles of 3-*iodo*-L-*thyronine* rapidly separated; they had m. p. 253° (decomp.), $[\alpha]_D^{21}$ +24·9° [Found: C, 43·3; H, 4·0; I, 30·0; loss of wt. (over $P_2O_5$ for 10 hr. at 120°/0·05 mm.), 3·5. $C_{15}H_{14}O_4NI,H_2O$ requires C, 43·2; H, 3·9; I, 30·4; $H_2O$, 4·3%].

3,3′,5′-*Tri-iodo*-L-*thyronine*.—3-Iodo-L-thyronine (834 mg.) was treated in 33% aqueous diethylamine (9 ml.) with a solution of iodine (1170 mg.) and potassium iodide (3·0 g.) in water (4 ml.), with stirring, during 20 min. The brown solution was shaken for 15 min. and water (5 ml.) added. The pH was adjusted to 5 by 2N-hydrochloric acid and after refrigeration for 1 hr. the pale brown solid was collected, washed with water, and dissolved in cold ethanol (60 ml.) containing N-sodium hydroxide (6 ml.). After filtration the pH was adjusted to 5 by 10% acetic acid, and the solution shaken with charcoal and kieselguhr at room temperature for 15 min. The clear filtrate was treated slowly at the b. p. with hot water (300 ml.). Needles slowly separated, and the solution was cooled slowly, then refrigerated overnight, giving 3,3′,5′-*tri-iodo*-L-*thyronine* (882 mg., 66%), m. p. 206° (decomp.), $[\alpha]_D^{21}$ +16·7° (Found: C, 27·1; H, 2·1; N, 2·1; I, 56·2. $C_{15}H_{12}O_4NI_3,H_2O$ requires C, 26·9; H, 2·1; N, 2·1; I, 56·9%).

3,3′-*Di-iodo*-L-*thyronine*.—3-Iodo-L-thyronine (1·668 g.) was treated in 33% aqueous diethylamine (18 ml.) with iodine (1420 mg.) and potassium iodide (4·0 g.) in water (10 ml.) as above, and shaken for 1 hr. Water (10 ml.) was then added, and most of the diethylamine was removed under reduced pressure at 30°. The pH was adjusted to 5·6 by 2N-acetic acid, and the solid collected, washed with ice-water, and dried under reduced pressure, leaving a pale brown powder (2·425 g.). The crude di-iodothyronine was chromatographed in 1,1-dimethylpropan-1-ol saturated with 6N-ammonia (50 ml.) on Whatman standard-grade cellulose powder (60 g.; 3 × 32 cm.). Fractions were eluted with the same solvent and examined by paper chromatography, and the fractions which contained <1% of 3-iodothyronine and <12% of 3,3′,5′-tri-iodothyronine (1·26 g. in all) were pooled and rechromatographed on an identical column. 3,3′-*Di-iodo*-L-*thyronine* containing <1% each of 3-iodothyronine and 3,3′,5′-tri-iodothyronine was obtained in 45% yield (from 3-iodo-L-thyronine) as needles, m. p. 196° (decomp.), $[\alpha]_D^{20}$ +18·8° (Found: N, 2·7; I, 45·6. $C_{15}H_{13}O_4NI_2,2H_2O$ requires N, 2·5; I, 45·3%). The amino-acid can be crystallized from 80% methanol (5 ml./g.) in 70% yield.

[17] Covert and Adkins, *J. Amer. Chem. Soc.*, 1932, **54**, 4116.

3′,5′-*Di-iodo*-L-*thyronine*.—L-Thyronine (5·46 g.) was treated in 33% aqueous ethylamine (1100 ml.) with iodine (17·7 g.) and potassium iodide (47 g.) in water (310 ml.) as above during 1 hr. The solution was set aside for 1 hr. longer, then most of the ethylamine was removed under reduced pressure. The pH was adjusted to 6·1 by 20% acetic acid, and the yellow solid collected. It was dissolved in ethanol (150 ml.) containing 2N-hydrochloric acid (25 ml.), and charcoal (2 g.) was added to the deep orange solution. After being shaken in the cold for 1 hr. and filtered, the solution was again treated with charcoal, giving an almost colourless filtrate, which was taken to pH 5 by dilute ammonia and concentrated to about 200 ml. under reduced pressure. The mixture was refrigerated for 1 hr. and the pale yellow solid was collected, washed with water several times, and dried, leaving a pale buff solid, 6·72 g. (64%), m. p. 198° (decomp.).

This solid was ground and then shaken for 2 hr. with 0·1N-sodium carbonate. The cloudy liquid was decanted from a dark residue (970 mg.), then shaken with kieselguhr and filtered, and the pH was adjusted to 5·5 in the cold with 2N-acetic acid. The almost colourless 3′,5′-di-iodothyronine was dried at room temperature in a neutral atmosphere, leaving 4·23 g. (40%) of material, m. p. 198° (decomp.), which contained at least 5% of 3′-iodothyronine. It was boiled with 2N-hydrochloric acid (250 ml.), and the almost colourless crystals were collected from the boiling solution and washed with more boiling 2N-hydrochloric acid (20 ml.). The residual hydrochloride was dissolved in cold 0·1N-hydrochloric acid (100 ml.) and ethanol (50 ml.). Cold saturated sodium acetate was added until the pH was about 5. 3′,5′-*Di-iodo*-L-*thyronine* rapidly separated as colourless crystals (3·40 g., 32%), m. p. 205° (decomp.), $[\alpha]_D^{21}$ +10·5° (Found: C, 33·4; H, 3·1; N, 2·7; I, 46·4. $C_{15}H_{13}O_4NI_2,H_2O$ requires C, 33·2; H, 2·8; N, 2·6; I, 46·7%). This material contained less than 2% of 3′-iodo-L-thyronine. Roche *et al.*,[3] and Block and Powell,[8] report m. p. 207° for 3′,5′-di-iodo-DL-thyronine.

3′-*Iodo*-L-*thyronine*.—L-Thyronine (2·73 g.) was treated in 33% aqueous ethylamine (550 ml.) with iodine (3·81 g.) and potassium iodide (8 g.) in water (75 ml.) with stirring, during 30 min. at room temperature. The pale brown solution was left for 1 hr. and most of the ethylamine was removed under reduced pressure. The pH was adjusted to 6 with 2N-hydrochloric acid at 5°, then the solution was refrigerated for 1 hr. and the solid collected. The mixed amino-acids were dissolved in 2N-hydrochloric acid (200 ml.). The solution deposited a dark liquid, which later solidified. The mixture was shaken for 2 hr., and the solid (1·91 g.), which contained about 50% each of 3′-iodo- and 3′,5′-di-iodo-L-thyronine, was filtered off, and the filtrate taken to pH 6 by 2N-ammonia, giving 3′-iodothyronine (1·76 g.) which contained about 8% of 3′,5′-di-iodothyronine. The residue was extracted again with 2N-hydrochloric acid (50 ml.), leaving a solid which contained about 70% of 3′,5′-di-iodo-L-thyronine and about 30% of 3′-iodo-L-thyronine. The filtrate on neutralization gave 3′-iodo-L-thyronine (0·28 g.) which contained less than 5% of 3′,5′-di-iodo-L-thyronine. The two crops of 3′-iodo-L-thyronine were combined, treated with cold 10N-hydrochloric acid (25 ml.), diluted to 125 ml. with water, and shaken for 2 hr. The precipitate was filtered off, and the filtrate, when taken to pH 6, deposited 3′-*iodo*-L-*thyronine* (1·50 g., 30%) as off-white needles, m. p. 207° (decomp.), $[\alpha]_D^{21}$ +15·7° (Found: C, 42·1; H, 4·5; N, 3·4; I, 30·0. $C_{15}H_{14}O_4NI,2H_2O$ requires C, 41·4; H, 4·2; N, 3·2; I, 29·2%). This material contained less than 2% of 3′,5′-di-iodo-L-thyronine. Roche *et al.*[3] report the same m. p. for 3′-iodo-DL-thyronine.

GLAXO LABORATORIES, LTD.,
GREENFORD, MIDDLESEX.

[*Received, January 7th,* 1960.]