169. *The Synthesis of Thyroxine and Related Substances. Part VI. The Preparation of Some Derivatives of* DL-*Thyroxine.*

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A number of new derivatives of DL-thyroxine have been prepared; their solubilities and physiological activities are recorded. A new method of iodination of 3 : 5-di-iodothyronine and its derivatives is described.

In the introduction to this series (Borrows, Clayton, and Hems, *J.*, 1949, S 185) it was stated that one of the aims of the work was to prepare derivatives of thyroxine that would be suitable for oral administration in the treatment of hypothyroidism. It was generally thought at that time (Harington, "The Thyroid Gland," London, 1933) that free thyroxine was ineffective orally, presumably because of its low solubility and consequent poor absorption from the gut. If that were so a soluble derivative might be more useful. We have prepared certain new derivatives of thyroxine; values have been obtained for their solubilities and physiological potencies, and are shown in the Table. Thyroxine methyl ester is included

Substance.	Activity.	Solubility in water, (g./100 ml. at 25°).
DL-Thyroxine monosodium salt	1·0	<0·001 pH 7·0
Thyroxine methyl ester	0·65	0·017 pH 7·6
Thyroxine ethyl ester	0·5	0·002 pH 7·6
N-Formylthyroxine	0·3	0·16 pH 7·3 0·37 pH 9·2
N-Phthaloylthyroxine	inactive	<0·02 pH 7·6
N-β-Carboxypropionylthyroxine	0·07	0·95 pH 7·6
N-Oxalothyroxine	0·3	0·14 pH 7·6
O-Carboxymethylthyroxine	0·1	0·03 pH 7·6
N-Carbamylthyroxine sodium salt	inactive	0·6 pH 7·0
Thyroxine methyl ether	0·5	<0·008 pH 7·6

since no figure for its activity has been reported. The biological activities of these compounds were determined by injection into mice, using the method devised by Smith, Emmens, and Parkes (*J. Endocrinol.*, 1947, **5**, 186).

Thyroxine ethyl ester hydrochloride was prepared by treating with hydrogen chloride a suspension of the amino-acid in ethyl alcohol: it was converted into the *ethyl* ester by treatment with the equivalent quantity of sodium hydroxide solution. N-*Formylthyroxine* was prepared by heating the amino-acid with formic acid under reflux, and N-*phthaloylthyroxine* was obtained by treatment of thyroxine with phthalic anhydride at 180° according to Billmann and Harting's general method (*J. Amer. Chem. Soc.*, 1948, **70**, 1473). For other *N*-acyl derivatives the methyl ester of thyroxine in anisole solution was treated with the appropriate acid chloride and the *N*-acyl esters were then hydrolysed with sodium hydroxide solution (cf. Ashley and Harington, *Biochem. J.*, 1928, **22**, 1436). Thus, N-β-*carboxypropionylthyroxine* and N-*oxalothyroxine* were prepared from thyroxine methyl ester and β-carbethoxypropionyl chloride and methoxalyl chloride, respectively.

When thyroxine was heated under reflux with 2N-sodium hydroxide solution and chloroacetic acid, the substance obtained on acidification appeared to be O-*carboxymethylthyroxine*, since it showed a positive ninhydrin reaction, but no colour with sodium nitrite in acid solution followed by basification with ammonia, a test in which 2 : 6-di-iodophenols give a red colour (Kendall and Osterberg, *J. Biol. Chem.*, 1919, **40**, 265; Harington and Barger, *Biochem. J.*, 1927, **21**, 169). Furthermore, electrometric titration demonstrated the presence of one basic group and only two acidic groups.

The monosodium salt of N-*carbamylthyroxine* was prepared by heating the sodium salt of thyroxine with an aqueous solution of sodium cyanate. Attempts to prepare the free hydantoic acid from the sodium salt failed, since rapid cyclisation to *thyroxinehydantoin* took place. This

last compound was characterised by catalytic hydrogenolysis, the product proving identical with *thyroninehydantoin* prepared from thyronine by the usual method.

There is some doubt about the activity of thyroxine methyl ether, which has been prepared by two different methods. One was the action of diazomethane on thyroxine, followed by hydrolysis of the resultant ester (Loeser, U.S.P. 2,252,230); the product was reported to melt at 226—228° and was claimed to be highly active. In the other, *N*-acetylthyroxine was methylated with methyl sulphate, and the acetyl group was subsequently removed by hydrolysis (Myers, *Proc. Staff Meetings, Mayo Clinic*, 1932, **7**, 201; *J. Amer. Chem. Soc.*, 1932, **54**, 3718). The product melted at 210—213° and was said to be active when administered to tadpoles, but had no effect upon normal men. Difficulties were encountered in attempting to follow Myers's intructions and no methyl ether was obtained. Consequently, thyroxine methyl ether was prepared by Loeser's procedure and identified as the methyl ether by hydrogenolysis to a compound that proved identical with an authentic specimen of *thyronine methyl ether*; this was prepared by hydrolysis and simultaneous racemisation of 3 : 5-di-iodo-4-*p*-methoxyphenoxy-*N*-acetyl-L-phenylalanine ethyl ester to 3 : 5-*di-iodo*-4-p-*methoxyphenoxy*-DL-*phenylalanine*, which was then hydrogenolysed.

In preparing thyroxine by the iodination of 3 : 5-di-iodothyronine in strong aqueous ammonia (Harington and Barger, *loc. cit.*; Harington, *Biochem. J.*, 1928, **22**, 1429) trouble was often encountered through the formation of explosive nitrogen iodides. By careful working, in particular by adding the iodine solution slowly below the surface of the liquid, the formation of nitrogen iodides was avoided, but the method was thought to involve an undesirable risk, particularly in larger scale preparations. To avoid this risk several other known methods of iodination were attempted, but none was satisfactory. Finally it was thought that solutions of organic bases might be useful as solvents, since their iodine derivatives are known to be non-explosive. The use of tertiary bases met with no success, but aqueous or alcoholic solutions of primary or secondary aliphatic bases proved to be very suitable media for iodination. 3 : 5-Di-iodothyronine is much more soluble in these solutions than in aqueous ammonia, and the iodination appeared to proceed more rapidly, although detailed studies on this point have not been carried out. The method is simple and convenient and thyroxine is obtained consistently in 90% yield. Several primary and secondary aliphatic bases have been used and aqueous ethylamine solutions have proved to be generally useful. The method has also been used in the iodination of derivatives of 3 : 5-di-iodothyronine, *N*-formyl- (Harington, *loc. cit.*), N-*acetyl*-, and N-β-*carboxypropionyl*-3 : 5-*di-iodothyronine*, giving the corresponding thyroxine derivatives in good yield. Similarly, 3 : 5-*di-iodothyroninehydantoin* could be iodinated to thyroxinehydantoin, which proved to be identical with the compound prepared by cyclisation of *N*-carbamylthyroxine.

Although iodination of 3 : 5-di-iodothyronine methyl ester did take place in aqueous solutions of bases, the compound underwent hydrolysis and thyroxine was the sole product. However, under anhydrous conditions, for example, using methyl alcohol and *n*-butylamine as the solvent, thyroxine methyl ester was readily obtained.

Experimental.

Thyroxine Ethyl Ester.—Hydrogen chloride was passed into a mixture of thyroxine (1 g.) and ethyl alcohol (10 ml.), and the solid rapidly went into solution. When the solution was saturated it was allowed to cool and was re-treated with hydrogen chloride. Evaporation yielded a solid residue of the ester hydrochloride (0·97 g.) which was dissolved in aqueous ethyl alcohol; one equivalent of N-sodium hydroxide was then added. The *ethyl* ester was precipitated as a white solid and was filtered off, washed, and dried. It (0·7 g.) had m. p. 156—157° (Found : C, 26·0; H, 1·8; N, 1·75; I, 62·55. $C_{17}H_{15}O_4NI_4$ requires C, 25·4; H, 1·9; N, 1·7; I, 63·1%).

N-*Formylthyroxine.*—Thyroxine (1 g.) was heated under reflux with formic acid (12 ml.; 98—100%) for five hours, and the solution cooled and evaporated *in vacuo* to dryness. The residue was heated for 1·5 hours with more formic acid (10 ml.), and the solution evaporated. The product was completely soluble in dry acetone from which it crystallised on addition of water. The material was recrystallised from aqueous acetone and the N-*formyl* compound separated as a white powder, m. p. 223° (decomp.) (Found : C, 25·2; H, 1·8; N, 1·6; I, 62·0. $C_{16}H_{11}O_5NI_4,\frac{1}{3}C_3H_6O$ requires C, 24·75; H, 1·6; N, 1·7; I, 61·7%).

N-*Phthaloylthyroxine.*—A mixture of thyroxine (0·5 g.) and phthalic anhydride (0·4 g.) was heated in a test-tube to 180°, by means of an oil-bath. The bath was maintained at this temperature for fifteen minutes. The solid slowly melted to a homogeneous brown liquid. After cooling, the solidified mass was extracted with boiling glacial acetic acid, and the solid obtained on cooling was recrystallised from ethyl acetate–light petroleum (b. p. 80—100°) as white prisms (0·3 g.), m. p. 156—157° (decomp.) (Found : C, 30·7; H, 1·8; N, 1·5. $C_{23}H_{13}O_6NI_4$ requires C, 30·45; H, 1·4; N, 1·5%).

N-*β-Carboxypropionylthyroxine.*—To a solution of thyroxine methyl ester (Ashley and Harington, *Biochem. J.*, 1928, **22**, 1436) (1 g.) in anisole (40 ml.) cooled to 0°, a solution of β-carbethoxypropionyl

chloride (0·14 g.) (Papa, Schwenk, and Hankin, *J. Amer. Chem. Soc.*, 1947, **69**, 3018) in anisole (2 ml.) was added. After one hour the precipitate was filtered off and identified by m. p. and mixed m. p. as thyroxine methyl ester hydrochloride (0·55 g.). The filtrate was evaporated to dryness *in vacuo* and the residue dissolved in benzene. After a short time white prisms (0·4 g.), m. p. 139—141°, were deposited, and were identified by analysis as N-*β-carbethoxypropionylthyroxine methyl ester* (Found: C, 29·1; H, 2·7; N, 1·4; I, 56·0. $C_{22}H_{21}O_7NI_4$ requires C, 28·7; H, 2·3; N, 1·5; I, 55·3%).

The ester was dissolved in ethyl alcohol (5 ml.) containing N-sodium hydroxide (4 ml.). After one hour at room temperature, N-hydrochloric acid (4 ml.) was added to precipitate the N-*β-carboxypropionylthyroxine*. This crystallised from aqueous alcohol as white prisms (0·25 g.), m. p. 202—203° (decomp.) (Found: C, 25·9; H, 1·5; N, 1·4; I, 57·5. $C_{19}H_{15}O_7NI_4$ requires C, 26·0; H, 1·7; N, 1·6; I, 58·0%).

N-*Oxalothyroxine*.—In a similar manner N-*oxalothyroxine* was prepared from thyroxine methyl ester and methoxalyl chloride; it crystallised from alcoholic alkali, on addition of acetic acid, as a white powder, m. p. 225—226° (decomp.) (Found: C, 24·2; H, 1·7; N, 1·6; I, 62·4. $C_{17}H_{11}O_7NI_4$ requires C, 24·05; H, 1·3; N, 1·65; I, 59·8%).

O-*Carboxymethylthyroxine*.—To thyroxine (1 g.) in water (30 ml.) containing N-sodium hydroxide (6 ml.) chloroacetic acid (0·12 g.) was added, and the mixture was boiled under reflux for two hours. An equal volume of alcohol was added and the mixture was treated with charcoal and filtered, and the pH adjusted to 5 by addition of glacial acetic acid. On cooling, silvery plates were deposited which were recrystallised by solution in alcoholic alkali and addition of acetic acid. O-*Carboxymethylthyroxine* (0·7 g.) had m. p. 208—209° (decomp.) (Found: C, 24·2; H, 2·1; N, 1·6; I, 60·1. $C_{17}H_{13}O_6NI_4$ requires C, 24·4; H, 1·6; N, 1·7; I, 60·9%).

Sodium Salt of N-*Carbamylthyroxine*.—Thyroxine monosodium salt (1 g.) suspended in water (75 ml.) containing sodium cyanate (0·4 g.) was heated under reflux for one hour and then an aqueous solution (25 ml.) of sodium cyanate (0·4 g.) was added and heating continued for three hours. The clear solution was concentrated *in vacuo* to 50 ml. and kept in the ice-chest whereupon the *sodium* salt separated as a white powder (0·7 g.). It was purified by dissolution in methyl alcohol (10 ml.) and addition of ether (100 ml.) whereupon a white solid was obtained (Found: N, 3·3; I, 59·5; Na, 2·9. $C_{16}H_{11}O_5N_2I_4Na$ requires, N, 3·3; I, 60·3; Na, 2·7%).

5-[3 : 5-*Di-iodo*-4-(3 : 5-*di-iodo*-4-*hydroxyphenoxy*)*benzyl*]*hydantoin*.—To a suspension of thyroxine monosodium salt (0·8 g.) in water (75 ml.) 0·2N-sodium hydroxide (8 ml.) and sodium cyanate (0·4 g.) were added and the solution was heated under reflux for 2·5 hours. Concentrated hydrochloric acid (40 ml.) was added, and heating was continued for one hour. After cooling, the *hydantoin* was filtered off and recrystallised from acetic acid from which it separated as white prisms (0·73 g.), softening at 169—170° and decomposing at 235° (Found: C, 25·4; H, 1·6; N, 3·4. $C_{16}H_{10}O_4N_2I_4,C_2H_4O_2$ requires C, 25·1; H, 1·6; N, 3·25%).

5-(4-p-*Hydroxyphenoxybenzyl*)*hydantoin*.—(*a*) A suspension of thyronine (2 g.) in water (20 ml.) containing sodium cyanate (1 g.) was refluxed until all solid had dissolved. Concentrated hydrochloric acid (12 ml.) was added and the mixture was heated under reflux for a further 30 minutes. The solid obtained on cooling was filtered off and recrystallised from aqueous acetic acid from which the *hydantoin* separated as colourless feathery clusters (1·9 g.), m. p. 256—257° (decomp.) (Found: C, 64·0; H, 4·8; N, 9·3. $C_{16}H_{14}O_4N_2$ requires C, 64·4; H, 4·7; N, 9·4%).

(*b*) 5-[3 : 5-Di-iodo-4-(3 : 5-di-iodo-4-hydroxyphenoxy)benzyl]hydantoin (0·17 g.) was hydrogenolysed in 0·1N-sodium hydroxide solution (20 ml.) using palladised calcium carbonate (0·5 g.) as catalyst. The crude product was crystallised from aqueous acetic acid and proved identical [m. p. and mixed m. p. 256—257° (decomp.)] with a specimen of the material obtained in (*a*) above (yield, 0·055 g.).

Thyroxine Methyl Ether.—This compound was prepared from thyroxine methyl ester by treatment with diazomethane and hydrolysis (Loeser, U.S.P. 2,252,230). It had m. p. 224—225° (decomp.).

3 : 5-*Di-iodo*-4-p-*methoxyphenoxy*-DL-*phenylalanine*.—A mixture of 3 : 5-di-iodo-4-*p*-methoxyphenoxy-*N*-acetyl-L-phenylalanine ethyl ester (see Part V, *J.*, 1949, 3424) (2 g.) and 2N-sodium hydroxide solution (30 ml.) was heated under reflux for 24 hours. To the cooled solution, acetic acid was added whereupon a white solid was deposited. This was filtered off and recrystallised from 50% aqueous acetic acid, from which the *phenylalanine* separated as white prisms, m. p. 238—239° (decomp.) (Found: N, 2·3; I, 46·9. $C_{16}H_{15}O_4NI_2$ requires N, 2·6; I, 47·1%).

Thyronine Methyl Ether.—(*a*) A solution of 3 : 5-di-iodo-4-*p*-methoxyphenoxyphenylalanine (0·6 g.) in ethyl alcohol (10 ml.) and 2N-sodium hydroxide (10 ml.) was shaken in hydrogen with palladised calcium carbonate (0·5 g.) until the uptake of hydrogen was complete. The filtered solution was acidified with acetic acid and, on storage, colourless needles of *thyronine methyl ether* separated and were crystallised from aqueous ethyl alcohol. This ether had m. p. 248° (decomp.) (Found: C, 66·8; H, 6·2; N, 5·0. $C_{16}H_{17}O_4N$ requires C, 66·9; H, 5·9; N, 4·9%).

(*b*) A solution of thyroxine methyl ether (0·4 g.) in ethyl alcohol (8 ml.) and 2N-sodium hydroxide (8 ml.) was shaken in hydrogen with palladised calcium carbonate (0·5 g.) until hydrogenolysis was complete. The solution was filtered and acidified with acetic acid; a white solid, m. p. 246° (decomp.) undepressed on admixture with a sample of thyronine methyl ether from (*a*) above, was precipitated.

Iodination of 3 : 5-*Di-iodothyronine and Its Derivatives*.—*Thyroxine*. To a stirred solution of 3 : 5-di-iodothyronine (2 g.) in aqueous ethylamine solution (20 ml.; 20%) a solution of iodine in potassium iodide solution (8·2 ml.; 1·85N.) was added dropwise. After being stirred for ten minutes the mixture was acidified with glacial acetic acid, and the precipitated thyroxine was filtered off and redissolved in ethyl alcohol (20 ml.) and 2N-sodium hydroxide solution (10 ml.). The hot solution was acidified with acetic acid; the thyroxine separated as a microcrystalline powder (2·6 g.; 90%), m. p. 231—232° (decomp.).

3 : 5-*Di-iodo*-N-*acetylthyronine*. To a solution of 3 : 5-di-iodothyronine methyl ester (1 g.) in anisole (30 ml.), a solution of acetyl chloride (0·072 g.) in anisole (2 ml.) was added. Later the precipitate was filtered off and identified by m. p. and mixed m. p. [227° (decomp.)], as 3 : 5-di-iodothyronine methyl ester hydrochloride (0·54 g.). The filtrate was evaporated to dryness *in vacuo* and the residue was

dissolved in ethyl alcohol (5 ml.) and N-sodium hydroxide (5 ml.). After one hour N-hydrochloric acid (5 ml.) and water (30 ml.) were added to precipitate a white solid which was recrystallised from glacial acetic acid. It formed white prisms, m. p. 207—208° (0·35 g.) (Found: C, 36·0; H, 2·65; N, 2·2; I, 44·8. $C_{17}H_{15}O_5NI_2$ requires C, 36·0; H, 2·7; N, 2·5; I, 44·8%).

3 : 5-*Di-iodo*-N-*β-carboxypropionylthyronine*. To a solution of 3 : 5-di-iodothyronine methyl ester (1 g.) in anisole (20 ml.) a solution of β-carbethoxypropionyl chloride (0·07 g.) in anisole (2 ml.) was added. The precipitated 3 : 5-di-iodothyronine methyl ester hydrochloride was filtered off, and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in ethyl alcohol (5 ml.) and N-sodium hydroxide (5 ml.). After one hour N-hydrochloric acid (5 ml.) was added and the solid filtered off. It was dissolved in alcoholic alkali, treated with charcoal, and reprecipitated with acid to give tiny white prisms of 3 : 5-*di-iodo*-N-*β-carboxypropionylthyronine* (0·3 g.), m. p. 231—232° (decomp.) (Found: N, 2·2; I, 41·2. $C_{19}H_{17}O_7NI_2$ requires N, 2·2; I, 40·7%).

Using the above method of iodination *N*-formyl-, *N*-acetyl-, and *N*-β-carboxypropionyl-thyroxine and 5-[3 : 5-di-iodo-4-(3 : 5-di-iodo-4-hydroxyphenoxy)benzyl]hydantoin were prepared from the corresponding derivatives of 3 : 5-di-iodothyronine and 5-(3 : 5-di-iodo-4-*p*-hydroxyphenoxybenzyl)-hydantoin.

Thyroxine methyl ester. To a solution of 3 : 5-di-iodothyronine methyl ester (1 g.) in *n*-butylamine (10 ml.) and methyl alcohol (20 ml.) a solution of iodine (1 g.) in methyl alcohol (10 ml.) was added. After one hour methanolic hydrogen chloride was added until the reaction solution was acid; dilution with water containing a little sodium acetate precipitated the crude ester which was extracted with methyl alcohol to give the pure compound (0·7 g.), m. p. and mixed m. p. 158°.

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