XII. THE CONFIGURATIVE RELATIONSHIP OF THYROXINE AND TYROSINE.

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SINCE the constitution of thyroxine has been known it has been generally assumed that the compound is formed in the organism by the oxidative coupling of two molecules of 3 : 5-diiodotyrosine. The likelihood of such a reaction was indeed already referred to by Harington and Barger [1927] in the consideration of the then unknown orientation of the iodine atoms in the thyroxine molecule, and the hypothesis was considerably strengthened by the demonstration by Harington and Randall [1929] of the actual occurrence of 3 : 5-diiodotyrosine side by side with thyroxine in the thyroid gland.

Search which has been made in this and other laboratories for the possible existence of an enzymic mechanism capable of converting 3 : 5-diiodotyrosine into thyroxine has hitherto proved fruitless, so that direct biochemical evidence of the relationship of the two compounds is still lacking; nor is the hypothesis as yet susceptible of proof by biological means. There remains however a line of chemical investigation which is described in the present paper and of which the results appear to be conclusive.

It is evident that if thyroxine is in fact synthesised from 3 : 5-diiodotyrosine under biological conditions it must be configuratively related to the latter amino-acid and therefore also to tyrosine from which diiodotyrosine is undoubtedly derived. A chemical synthesis of thyroxine from natural *l*-tyrosine conducted by a series of reactions involving no racemisation and no possibility of a Walden inversion at any stage should lead therefore to the naturally occurring modification of thyroxine.

Unfortunately a direct synthesis of thyroxine itself from tyrosine or 3 : 5-diiodotyrosine is not feasible; on the one hand the halogenation of the thyronine nucleus in the 3 : 5-positions cannot be accomplished and on the other it is impossible to carry out the phenyl ether condensation with a phenolic substance such as 3 : 5-diiodotyrosine which is substituted in the *ortho*-positions to the hydroxyl group.

The problem has therefore had to be solved in an indirect manner. By a series of reactions to be described below it has been possible, starting from natural *l*-tyrosine, to synthesise an optically active thyronine having $[\alpha]_{5461} + 13.3^\circ$. Now we had in our hands some *l*-thyroxine which had been prepared by the resolution of *dl*-thyroxine according to Harington [1928] and which had been shown by the work of Harington and Salter [1930] to be identical with the naturally occurring compound; catalytic deiodination of a sample of this *l*-thyroxine yielded a preparation of thyronine having $[\alpha]_{5461} + 12.2^\circ$.

There can thus be no longer any doubt of the stereochemical relationship of natural *l*-thyroxine with natural *l*-tyrosine, and on chemical grounds at least we may feel secure in advancing the hypothesis that in nature thyroxine is derived from tyrosine.

Synthesis of d-thyronine.

The actual synthesis of *d*-thyronine from *l*-tyrosine was carried out as follows. The ethyl ester of *N*-benzoyl-*l*-tyrosine was condensed with 3 : 4 : 5-triiodonitrobenzene by boiling in methylethylketone solution with potassium carbonate to give ethyl α -benzamido- β -[4-(3' : 5'-diiodo-4'-nitrophenoxy)phenyl]-propionate (I). (The object of employing 3 : 4 : 5-triiodonitrobenzene instead of the more

obvious *p*-iodonitrobenzene is simply to improve the yield at the stage of the condensation; the subsequent removal of the 3' : 5'-iodine atoms offers no difficulties.) The ester (I) was hydrolysed to the free acid, which in turn was reduced with ferrous sulphate and baryta to the corresponding amino-compound (II); the latter was deiodinated by catalytic hydrogenation in presence of palladised calcium carbonate, and the resulting iodine-free amino-acid (III) was diazotised in dilute hydrochloric acid solution. The *N*-benzoylthyronine, which resulted on boiling the solution of the diazonium salt, was not isolated but was converted directly into *d*-thyronine (IV) by further boiling after the concentration of hydrochloric acid had been increased to 5*N*.

As already mentioned the thyronine obtained in this way had $[\alpha]_{5461} + 13.3^\circ$, whilst a sample of thyronine obtained by the catalytic deiodination of *l*-thyroxine ($[\alpha]_{5461} - 3.5^\circ$) had $[\alpha]_{5461} + 12.2^\circ$; a third sample obtained by deiodination of *l*-3 : 5-diiodothyronine (itself related to *l*-thyroxine) had $[\alpha]_{5461} + 13.9^\circ$.

EXPERIMENTAL.

N-benzoyl-*l*-tyrosine ethyl ester. This compound was most conveniently prepared as follows. *l*-Tyrosine ester hydrochloride (24.5 g.) was dissolved in the minimum amount of water; chloroform (200 ml.) was then added and the

mixture cooled to -10° . The free ester was liberated by addition of 5*N* sodium hydroxide (20 ml.) with vigorous shaking. Benzoyl chloride (14 g.) was then added in portions with shaking after each addition. The mixture was treated with 2*N* sodium carbonate (50 ml.) followed by a further equal amount of benzoyl chloride in portions. When evolution of CO_2 no longer occurred on shaking, the chloroform layer was separated, washed with water, dried over calcium chloride and evaporated; recrystallisation of the residue from a mixture of chloroform and light petroleum afforded 85 % of the theoretical amount of a product having m.p. 119° .

Ethyl α -benzamido- β -[4-(3':5'-diiodo-4'-nitrophenoxy)phenyl]propionate (I). *N*-benzoyl-*L*-tyrosine ethyl ester (20 g.: 15 % excess) and 3:4:5-triiodonitrobenzene (28 g.) were dissolved in methylethylketone (200 ml.); freshly dried and finely powdered potassium carbonate (8.7 g.) was added and the mixture boiled under reflux for 18 hours. The solution was filtered and the precipitate extracted with hot methylethylketone; the combined filtrates were evaporated on the water-bath to a pasty residue which was triturated with cold water. The insoluble portion which was somewhat sticky was collected and recrystallised from 95 % alcohol (1800 ml.). The yield was 17.2 g. (45 % of the theoretical).

After recrystallisation from acetic acid the compound formed colourless prismatic needles, m.p. 189° . It had $[\alpha]_{5461} - 1.8^{\circ}$ ($c = 3.65$ in acetic acid; 2 dm. tube). (Found: N, 4.0; I, 37.3 %. $\text{C}_{24}\text{H}_{20}\text{O}_6\text{N}_2\text{I}_2$ requires: N, 4.1; I, 37.0 %.)

α -Benzamido- β -[4-(3':5'-diiodo-4'-nitrophenoxy)phenyl]propionic acid. The above ester (16 g.) was suspended in alcohol (55 ml.); 5.1*N* sodium hydroxide (5 ml.) was added and the mixture warmed on the water-bath with constant stirring; it became at first reddish in colour and more liquid; on further heating for a few minutes the colour disappeared and the consistency became thick and pasty. Water (200 ml.) was added and the mixture warmed until a clear solution was obtained; addition of hydrochloric acid precipitated the product, which was collected and crystallised from 70 % acetic acid. The yield was 14.7 g. and a further 0.75 g. was obtained by dilution of the mother-liquor with water.

For complete purification the acid was dissolved in methylethylketone and the filtered solution evaporated; the residue was then again crystallised from 70 % acetic acid. The compound formed colourless needles having m.p. 207° ; it had $[\alpha]_{5461} - 6.8^{\circ}$ ($c = 2.72$ in acetic acid; 2 dm. tube). (Found: N, 3.9; I, 38.4. $\text{C}_{22}\text{H}_{16}\text{O}_6\text{N}_2\text{I}_2$ requires: N, 4.3; I, 38.6 %.)

α -Benzamido- β -[4-(3':5'-diiodo-4'-aminophenoxy)phenyl]propionic acid (II). The nitro-acid (11.7 g.) was dissolved in water (120 ml.) with the aid of sodium hydroxide sufficient just to make the solution alkaline to phenolphthalein. The solution was added to a suspension of ferrous hydroxide prepared by mixing a solution of hydrated ferrous sulphate (30 g.) in water (150 ml.) with one of hydrated barium hydroxide (38 g.) in water (100 ml.); the mixture was heated on the water-bath for 10 minutes. It was then brought to the boil, treated with a slight excess of sodium sulphate and filtered hot; the precipitate was extracted with boiling water and the combined solutions (about 1 litre) acidified with acetic acid and allowed to cool. The first crop amounted to 7.2 g. and a further 2.2 g. was obtained by working up the mother-liquor; the total yield was therefore 84 % of the theoretical. For analysis the acid was recrystallised from 50 % alcohol (charcoal) from which it separated in colourless needles having m.p. 197° . It had $[\alpha]_{5461} - 15.0^{\circ}$ ($c = 2.47$ in a mixture of equal parts of *N* hydrochloric acid and alcohol; 2 dm. tube). (Found: N, 4.4; I, 39.8 %. $\text{C}_{22}\text{H}_{18}\text{O}_4\text{N}_2\text{I}_2$ requires: N, 4.5; I, 40.4 %.)

α -Benzamido- β -[4-(4'-aminophenoxy)phenyl]propionic acid (III). The preceding compound (9.75 g.) was dissolved in a mixture of *N* sodium hydroxide (280 ml.) and alcohol (200 ml.) and after addition of palladised calcium carbonate (15 g. [cf. Harington, 1926]) the solution was shaken in an atmosphere of hydrogen. Uptake of hydrogen ceased at 778 ml. (corrected; about 10 % in excess of the theoretical); the catalyst was filtered off and washed with 50 % alcohol. Alcohol was removed from the combined filtrates by distillation under diminished pressure and the aqueous residue acidified with acetic acid. The product was collected and dissolved in boiling water (200 ml.) with the aid of sodium acetate; the filtered solution was treated at the boiling-point with hydrochloric acid to give maximum precipitation. The yield was 5.1 g. (93 % of the theoretical).

For analysis the acid was recrystallised from 50 % alcohol; it formed colourless needles having m.p. 182.5°. It had $[\alpha]_{5461} - 40.0^\circ$ ($c = 2.62$ in a mixture of equal parts of *N* hydrochloric acid and alcohol; 2 dm. tube). (Found: N, 7.1 %; $C_{22}H_{20}O_4N_2$ requires: N, 7.4 %.)

d-Thyronine (IV). The compound III (1.6 g.) was dissolved in 0.3 *N* hydrochloric acid (42.5 ml.) with the aid of heat; on cooling, the solution remained clear. Diazotisation was effected by the gradual addition of powdered sodium nitrite (0.3 g.) the solution being stirred and maintained at 0° throughout and for 15 minutes after the last addition; the diazonium salt crystallised in part. The mixture was diluted with water (40 ml.), and stirring was continued at 0° for 15 minutes longer; the whole was then transferred to a flask and boiled under reflux for 1.5 hours. An equal volume of concentrated hydrochloric acid was added and boiling continued for 6 hours more to effect hydrolysis of the benzoyl group. After cooling, the solution was diluted with an equal volume of water and extracted with ether to remove benzoic acid. The aqueous layer was evaporated to dryness under diminished pressure, and the residue was taken up in water (about 20 ml.); treatment of the hot aqueous solution with sodium acetate caused precipitation of the amino-acid. The yield was about 0.5 g.

The product was purified by dissolution in dilute sulphuric acid and reprecipitation from the hot solution by addition of sodium acetate. It had m.p. 249° (decomp.) and the m.p. was not affected by admixture with *d*-thyronine obtained from *l*-thyroxine (see below). The amino-acid had $[\alpha]_{5461} + 13.3^\circ$ ($c = 0.75$ in a mixture of equal parts of *N* hydrochloric acid and alcohol; 2 dm. tube). (Found: N, 5.0 %. $C_{15}H_{15}O_4N$ requires: N, 5.1 %.)

d-Thyronine from *l*-thyroxine. *l*-Thyroxine (0.075 g.; $[\alpha]_{5461} - 3.5^\circ$) was dissolved in *N* potassium hydroxide (7.5 ml.) and shaken in an atmosphere of hydrogen after addition of palladised calcium carbonate (0.25 g.); at the conclusion of the uptake of hydrogen the solution was filtered, acidified with acetic acid and evaporated on the water-bath; the recrystallised thyronine amounted to 0.021 g. It had m.p. 247° (decomp.) and $[\alpha]_{5461} + 12.2^\circ$ ($c = 4.12$ in a mixture of equal parts of *N* hydrochloric acid and alcohol; 0.5 dm. micro-tube). (Found: N, 5.3 %. $C_{15}H_{15}O_4N$ requires: N, 5.1 %.)

d-Thyronine from *l*-3 : 5-diiodothyronine. For further confirmation a preparation of thyronine was made from *l*-3 : 5-diiodothyronine which is itself known to be stereochemically related to *l*-thyroxine [cf. Harington, 1928]. *l*-3 : 5-Diiodothyronine (0.3 g.; $[\alpha]_{5461} - 1.0^\circ$) was catalytically deiodinated in presence of palladised calcium carbonate (1 g.); the yield of thyronine was 0.128 g. and the product had m.p. 252° (decomp.) and $[\alpha]_{5461} + 13.9^\circ$ ($c = 0.753$ in a mixture of equal parts of *N* hydrochloric acid and alcohol; 2 dm. tube). (Found: N, 5.1 %. $C_{15}H_{15}O_4N$ requires: N, 5.1 %.)

Note on preparation of 1-4-(4'-aminophenoxy)phenylalanine.

α -Benzamido- β -[4-(4'-aminophenoxy)phenyl]propionic acid (III) (0.5 g.) was boiled under reflux for 4 hours with 20 % hydrochloric acid (10 ml.); the solution was cooled, diluted with water and extracted with ether to remove benzoic acid. The aqueous layer was evaporated to dryness under diminished pressure and the residue taken up in a little hot water. After partial neutralisation with sodium acetate the solution was boiled with charcoal and filtered and the filtrate carefully neutralised to litmus. The product which separated on cooling was collected and recrystallised from boiling water from which it separated in colourless glistening needles. It had m.p. 239° (decomp.) and $[\alpha]_{5461} - 5.0^{\circ}$ ($c = 1.2$ in 0.5 *N* hydrochloric acid; 1 dm. tube). The compound was considerably more soluble than thyronine in water; it gave a very intense ninhydrin reaction without previous neutralisation with sodium acetate. (Found: N, 10.3 %. $C_{15}H_{16}O_3N_2$ requires: N, 10.3 %.)

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