Synthesis of a Sulphur-containing Analogue of Thyroxine

By C. R. HARINGTON, *National Institute for Medical Research, London, N.W. 3*

(Received 5 April 1948)

It is generally accepted that the obvious physiological effects of the thyroid hormone, as manifested by its influence on the metabolic rate, are brought about by the peripheral action of the hormone on the tissues. Although it is still not completely certain that the circulating hormone is free thyroxine itself the balance of evidence points in this direction (Harington, 1944), and this supposition is strongly supported by the recent work of Taurog & Chaikoff (1947).

In addition to its effect in stimulating the metabolism of tissues, thyroxine has an action on the anterior pituitary gland in regulating the output of thyrotrophic hormone by the latter organ. It is this effect which is responsible for the hypertrophy of the thyroid gland that results from treatment with one of the antithyroid drugs of the thiouracil type; under the action of these drugs thyroxine synthesis in the thyroid gland is blocked, with resulting diminution of the concentration of circulating thyroid hormone in the body; the response of the anterior pituitary gland to this situation is to release more thyrotrophic hormone, which in turn produces overgrowth of the thyroid gland. Conversely, if circulating thyroid hormone is increased by exogenous administration, thyrotrophic hormone output is diminished, and the thyroid gland passes into a resting state.

The maintenance of normal conditions in the body, so far as thyroid hormone is concerned, must therefore depend on the precise relationship between the two actions of this hormone, namely its effect on tissue metabolism on the one hand and its effect on the anterior pituitary gland on the other. The question arises as to whether it is possible to dissociate these two effects.

If such a dissociation could be brought about the results would be of considerable interest. A compound possessing the characteristic effect of thyroxine on tissue metabolism but, lacking its action on the anterior pituitary gland would, if administered exogenously, produce a greatly exaggerated stimulation of metabolism, since its own intrinsic action in this direction would be unaccompanied by the restraint of thyroid gland activity imposed by diminution of thyrotrophic hormone output. On the other hand, a compound acting on the anterior pituitary gland in the same way as thyroxine, but lacking the power of the latter to stimulate tissue

metabolism, would bring about inhibition of thyroid activity and an overall reduction in the metabolic rate.

The latter possibility is the more interesting, for, with the aid of such a compound, thyroid activity could be controlled in a more rational manner than it can be by the so-called antithyroid drugs. The depression of thyroid function brought about by these drugs necessarily carries with it the likelihood of simultaneous production of physical hypertrophy of the gland; a compound having the properties discussed above should, however, diminish thyroid function, in the sense of thyroxine output, without entailing hypertrophy of the gland; its action should indeed cause a hyperactive gland, such as that existing in Graves's disease, to revert to the normal state. The therapeutic potentialities of such a compound are obvious.

Consideration on these lines points to the desirability of examining the physiological action of compounds closely allied in chemical composition to thyroxine itself. Certain substances of this kind are already known, namely the analogues of thyroxine in which the iodine atoms are replaced by bromine or chlorine (cf. Schuegraf, 1929). It was shown many years ago by Gaddum (1929-30) that 3:5:3':5'-tetrabromothyronine had the same qualitative effect as thyroxine, although in much lower degree. The action of this compound, and also that of the chlorine-containing analogue, have recently been investigated in human myxoedema (Lerman & Harington, 1948); both have proved to be capable, in appropriate doses, of restoring the metabolism of a myxoedematous patient to the normal level. In so far as these substances exhibit the characteristic peripheral effect of thyroxine, therefore, they do not seem to be promising from the present point of view; however, their action on the anterior pituitary gland remains to be investigated and the possibility exists that this may predominate over the peripheral effect; if such be the case it should be possible, by their administration at the correct level of dosage, to produce a diminution rather than an increase in the rate of metabolism.

In the meantime it has seemed desirable to make available for study quite a different type of thyroxine analogue and for this purpose the compound (I) was selected; it will be seen that this is identical with thyroxine save that sulphur replaces oxygen as

the element linking the two benzene rings. It appeared likely on general grounds that the sulphur-containing derivative could be synthesized on the same lines as thyroxine itself (cf. Harington & Barger, 1927). So far as the early stages of the

synthesis are concerned this indeed proved to be the case, and it was possible, starting with 4-methoxythiophenol and 3:4:5-triodonitrobenzene, to obtain 3:5-diiodo-4-(4'-methoxyphenylsulphidobenzaldehyde (II) by a series of reactions precisely similar to those

employed in the thyroxine synthesis; the yields in the diphenyl sulphide series were in fact rather better than in the diphenyl ether series.

Quite unexpectedly, however, the synthesis of 3:5-diiodo-4-(4'-hydroxyphenylsulphidophenylalanine (III) from the aldehyde (II) could not be carried out

by the modified Erlenmeyer method (cf. Harington & McCartney, 1927). Condensation of the aldehyde (II) with hippuric acid gave the *azlactone* in excellent yield, and this in turn could be smoothly converted into the corresponding *benzamidocinnamic acid*, but when either this compound or the *azlactone* itself was boiled with hydriodic acid, acetic acid and red phosphorus, elimination of the iodine atoms readily occurred and the product consisted of a mixture of partly and completely deiodinated material. Many attempts were made to circumvent this difficulty by modification of the conditions, but without success; even treatment with dilute hydriodic acid in acetic acid, as employed by Lamb & Robson (1931) in the stepwise conversion of *azlactones* and *benzamidocinnamic acids* into amino-acids, failed to give a satisfactory result.

In the case of diiodothyronine (Harington & McCartney, 1927) the analogous conversion of the *azlactone* or *benzamidocinnamic acid* can be carried out in good yield, although even here more recent experience has shown that too prolonged boiling with the hydriodic-acetic acid mixture and phosphorus causes more or less deiodination. It is clear, however, that the lability of the iodine atoms in the

corresponding diphenyl sulphide derivative is much greater, and an alternative route from the aldehyde to the amino-acid had to be found.

Success was attained quite simply as follows. The aldehyde (II) was reduced to the corresponding *alcohol* by means of aluminium isopropoxide; the alcohol was converted into the *chloride* and the latter condensed with ethyl acetamidomalonate; appropriate treatment of the resulting *ester* with a mixture of hydrobromic and acetic acids gave the hydroxy-diiodoamino-acid (III) in satisfactory yield and final iodination in ammoniacal solution afforded the desired thyroxine analogue (I).

In acute experiments the thyroxine analogue was non-toxic to mice in doses up to 0.5 g./kg. (subcutaneous) and 0.25 g./kg. (intravenous). Tested on tadpoles (*Xenopus laevis*) it accelerated metamorphosis, its activity in this respect being approximately one fifth of that of thyroxine. More detailed studies of the physiological action are now in progress.

EXPERIMENTAL

3:5-Diiodo-4-(4'-methoxyphenylsulphidobenzaldehyde. 3:4:5-Triiodonitrobenzene (86.5 g.) was dissolved in dioxan (175 ml.) and 4-methoxythiophenol (26 ml.) (Suter & Hanson, 1932) was added. The mixture was vigorously stirred at room temperature while NaOH (17.3 ml. of 10N) was run in. Heat was developed and crystalline material soon began to separate. Stirring was continued for 30 min. after which a sample diluted with water had a neutral reaction.

Water (about 2 vol.) was then added with further stirring; the precipitate, which was first oily but soon crystallized, was collected and washed with water. It was recrystallized from acetic acid, from which it separated in long bright yellow needles, m.p. 140°. The melting point was unchanged by further crystallization from methyl ethyl ketone. Yield 78 g. (92%). (Found: S, 6.2; I, 49.7. $C_{13}H_9O_3NSI_2$ requires S, 6.2; I, 49.5%.)

3:5-Diiodo-4-(4'-methoxyphenylsulphidoaniline. Stannous chloride (110 g. of the dihydrate) was suspended in acetic acid (500 ml.) and brought into solution by saturation with HCl while being stirred and heated to about 70° on a water bath. The above nitro compound (73.5 g.) was then added as a suspension in acetic acid (250 ml.). The water bath was brought to the boil, vigorous stirring and rapid introduction of HCl being continued. The reaction appeared to be complete in about 45 min. as shown by saturation with HCl and no further separation of stannichloride; after a further 15 min. the mixture was cooled and the stannichloride of the base collected and washed with cold acetic acid. It was then ground up and shaken well with NaOH solution (1500 ml. of 3N) and the base collected and washed with water. The base was dissolved in ether (3000–3500 ml.) and the ethereal solution washed with 200 ml. of 2N-NaOH, dried over NaOH pellets and saturated with HCl. The precipitated hydrochloride was collected, washed with ether and dried. The yield was 63 g. (84%). After recrystallization from ethanol containing a little HCl it had m.p. 166–168°. (Found: N, 2.5; S, 5.6. $C_{13}H_{11}ONSI_2 \cdot HCl$ requires N, 2.7; S, 6.2%.)

The free base was prepared by dissolving the hydrochloride in absolute ethanol, adding excess of ammonia to the hot solution and diluting with water. After recrystallization from butanol it formed colourless fern-shaped crystals, m.p. 177°. (Found: N, 2.7; S, 6.9. $C_{13}H_{11}ONSI_2$ requires N, 2.9; S, 6.6%.)

3:5-Diiodo-4(4'-methoxy)phenylsulphidobenzonitrile. A solution of cuprous cyanide was prepared by mixing NaCN (213 g.) dissolved in water (490 ml.) with $CuSO_4 \cdot 5H_2O$ (250 g.) in water (970 ml.). The hydrochloride of the above base (52 g.) was powdered and suspended in acetic acid (520 ml.); the stirred suspension was treated with amyl nitrite (15 ml.) which was added quickly; the solid passed into solution within a few minutes. The solution thus prepared was added slowly with stirring to the cooled cuprous cyanide and the mixture warmed to about 80° to complete the reaction. The precipitate was collected, washed with water and boiled out with three successive quantities of chloroform. The chloroform extracts were dried and evaporated to a low bulk, when crystallization set in. After thorough cooling the crystals were collected and recrystallized from methyl ethyl ketone, the solution being cooled in a freezing mixture. There was thus obtained 28.4 g. (55%) of a product, melting at 149° after some sintering, which was pure enough for the next reaction. After further recrystallization from methyl ethyl ketone the compound formed pale yellow hexagonal prisms, m.p. 150°. (Found: N, 2.5; S, 6.5; I, 51.4. $C_{14}H_9ONSI_2$ requires N, 2.8; S, 6.4; I, 51.5%.)

3:5-Diiodo-4(4'-methoxy)phenylsulphidobenzaldehyde (II). $SnCl_4$ (60 g.) was suspended in dry ether (220 ml.) and brought into solution by stirring and saturating with HCl. A solution of the above nitrile (28.15 g.) in dry chloroform (75 ml.) was added, stirring and introduction of HCl being continued for 5 hr. After standing overnight the mixture, which consisted of an upper pale yellow and a lower deep orange-coloured layer, was treated with crushed ice and evaporated *in vacuo* until all the organic solvents were removed. The solid residue was heated with dilute HCl to the boiling point and, after cooling, the crude aldehyde was taken up in chloroform; the chloroform solution was shaken with aqueous $Na_2S_2O_5$ (300 ml. of 30% (w/v)) and the bisulphite compound which separated was collected, washed with ether and decomposed by warming with dilute H_2SO_4 . The aldehyde was crystallized from acetic acid from which it separated in large pale yellow needles, m.p. 134° (unchanged by further recrystallization). The yield was 18.6 g. (66%). (Found: S, 6.3; I, 50.6. $C_{14}H_{10}O_2SI_2$ requires S, 6.45; I, 51.2%.)

Azlacone. The above aldehyde (11.1 g.), hippuric acid (4 g.) and fused sodium acetate (11.1 g.) were ground up together; the mixture was treated with acetic anhydride (70 ml.) and heated on the boiling water bath for 15 min. with intermittent stirring. After cooling, the mixture was stirred with a large volume of water containing some H_2SO_4 until the acetic anhydride was decomposed. The product was collected, washed with water and dried. The yield was 13.9 g. (97%) of practically pure azlacone, m.p. 224°. After recrystallization from acetic acid, in which it is very sparingly soluble, it formed yellow needles, m.p. 228°. (Found: C, 43.0; H, 2.35; S, 5.0. $C_{22}H_{15}O_3NSI_2$ requires C, 43.2; H, 2.5; S, 5.0%.)

3:5-Diiodo-4(4'-methoxy)phenylsulphidobenzamidocinnamic acid. The azlacone (9.5 g.) was added to a boiling mixture of ethanol (950 ml.) and 5N-NaOH (50 ml.); the solid passed

rapidly into solution and boiling was continued for 10 min. after this had happened. The solution was acidified while hot with concentrated HCl and diluted with an equal volume of water. This caused separation of the product in gelatinous form. The acid was brought into solution with ammonia and the boiling solution again acidified; boiling was continued for a few minutes after which the precipitated acid was almost completely crystalline. The yield of acid, collected after cooling, was quantitative. Recrystallized from aqueous dioxan it formed bunches of pale yellow needles, m.p. 245° (decomp.). (Found: S, 4.7. $C_{23}H_{17}O_6NSI_2$ requires S, 4.9%.)

As already indicated, all attempts to convert either this acid or the azlacone into 3:5-diiodo-4(4'-methoxy)phenylsulphidophenylalanine were frustrated by the ease with which the iodine atoms were eliminated on treatment with hydriodic acid and red phosphorus.

3:5-Diiodo-4(4'-methoxy)phenylsulphidobenzyl alcohol. 3:5-Diiodo-4(4'-methoxy)phenylsulphidobenzaldehyde (5 g.) was heated to gentle boiling under a reflux air condenser with a mixture of aluminium isopropoxide (20 ml. of approximately N solution in isopropanol), toluene (10 ml.) and isopropanol (10 ml.); a slow stream of N_2 was bubbled through the solution until evolution of acetone ceased (about 3½ hr.). The solution was evaporated to dryness *in vacuo* and the residue was ground up with dilute HCl, collected and washed with water. The moist filter cake was crystallized from acetic acid, in which it was very soluble; the product, which separated in colourless prismatic needles, m.p. 132°, amounted to 4.5 g. (90%). (Found: S, 6.5; I, 50.3. $C_{14}H_{12}OSI_2$ requires S, 6.4; I, 51.0%.)

3:5-Diiodo-4(4'-methoxy)phenylsulphidobenzyl chloride. The above alcohol (3 g.) was dissolved in warm dry chloroform (15 ml.). The solution was cooled in ice water and to the resulting crystalline paste PCl_5 (1.5 g.) was added. The mixture was shaken with continued cooling until practically all the solid had passed into solution; it was then kept at room temperature for 2 hr. A small amount of precipitate was removed by filtration and the filtrate was diluted with about 2 vol. of light petroleum (b.p. 60–80°); this produced a slight brown flocculum which was removed. The filtrate was then washed with water, $NaHCO_3$ solution (thoroughly) and again with water; it was dried over $CaCl_2$ and evaporated *in vacuo*. The residue crystallized almost at once; it was recrystallized from a small volume of acetic acid from which it separated in colourless rhombs, m.p. 110°. One further recrystallization raised the m.p. to 112°. The yield was 2.5 g. (83%). (Found: S, 6.3; I, 48.7. $C_{14}H_{11}OSClI_2$ requires S, 6.2; I, 49.1%.)

Ethyl 3:5-diiodo-4(4'-methoxy)phenylsulphidobenzylacetamidomalonate. A solution of sodium (0.1 g.) in ethanol (5 ml.) was prepared and, after cooling, ethyl acetamidomalonate (0.84 g.) was introduced; there was then added a solution of the above chloride (2 g.) in dioxan (5 ml.). The mixture was boiled under reflux; separation of NaCl soon began, and the reaction was complete in 2½ hr. On cooling and dilution with water an oily precipitate was produced which soon crystallized; this was collected, washed with water and recrystallized from ethanol. The ester formed colourless prismatic needles, m.p. 138–140°. The yield was 2.25 g. (83.5%). One further recrystallization from ethanol raised the m.p. to 144°. (Found: C, 39.8; H, 3.8; S, 4.5; I, 36.2. $C_{23}H_{25}O_6NSI_2$ requires C, 39.6; H, 3.6; S, 4.6; I, 36.4%.)

3:4 - *Diiodo-4(4'-hydroxy)phenylsulphidophenylalanine* (III). Prolonged boiling of the above ester with mixtures of hydrobromic and acetic acids caused considerable darkening with formation of tar and correspondingly poor yields of the desired amino-acid. It was assumed that of the three hydrolytic reactions occurring, deacetylation, de-esterification and demethylation, the last would be the slowest; an experiment was therefore carried out to determine the minimum time required for this reaction. In this experiment anisic acid was boiled under reflux with forty parts of a mixture of equal volumes of HBr (6N) and acetic acid, samples being withdrawn at intervals for estimation of the phenol (*p*-hydroxybenzoic acid) content. The results showed that demethylation was substantially complete (95–100%) in 2 hr.

The ester (6 g.) was therefore boiled under reflux for 2 hr. with a mixture of HBr (120 ml. of 6N) and acetic acid (120 ml.). The solution was evaporated to dryness *in vacuo* and the largely crystalline residue was boiled with water (about 250 ml.) and filtered hot through a wet paper to remove tar. The filtrate was brought to the boil and cautiously neutralized to about pH 5.0 with sodium acetate. The heavy granular precipitate which separated was collected after cooling, washed with water and redissolved in a mixture of 80 ml. ethanol and 20 ml. N-NaOH. On boiling this solution and adding acetic acid rapid separation of the amino-acid occurred in tufts of colourless needles, m.p. 262° (decomp.). The yield was 3.05 g. (65%). (Found: C, 33.0; H, 2.4; S, 6.1; I, 46.9. $C_{15}H_{11}O_3NSI_2$ requires C, 33.25; H, 2.4; S, 5.9; I, 47.0%.) The compound gave a ninhydrin reaction with a reddish hue, and a positive Millon reaction. It was practically insoluble in water and only very sparingly soluble in dilute aqueous mineral acids.

3:5 - *Diiodo-4(3':5'-diiodo-4'-hydroxy)phenylsulphidophenylalanine* (I). The diiodoamino-acid (3.6 g.) was dissolved in ammonia (144 ml. of sp.gr. 0.880); this solution was treated drop by drop with I_2 in KI (6.2 ml. of 4.31N). A small amount of tarry material separated towards the end of the reaction. After all the iodine had been added the solution was evaporated to dryness *in vacuo* and the residue, which was already partly crystalline, was dissolved in boiling 0.1N- Na_2CO_3 (about 1000 ml.); a little insoluble matter was removed by filtration and the filtrate was brought to about pH 5.0 at the boiling point by addition of dilute HCl. The partly crystalline precipitate was collected after cooling and dissolved in a mixture of 160 ml. ethanol and 40 ml. N-NaOH; the solution was

brought to the boil and cautiously treated with acetic acid. Just before neutrality was reached a pigmented flocculent precipitate separated; this was removed by filtration as rapidly as possible, the filtrate was brought to the boil and the acidification with acetic acid completed. The amino-acid separated in rosettes of tiny needles. The yield was 3.6 g. (68%). For complete purification the crystallization was repeated as above, again with removal of a slight pigmented flocculum, and the product was then obtained in sheaves of small but well-formed needles which were almost colourless; m.p. 226° (decomp.). (Found: C, 23.5; H, 1.6; S, 4.0; I, 64.0. $C_{15}H_{11}O_3NSI_4$ requires C, 22.7; H, 1.4; S, 4.0; I, 64.0%.) The compound resembled thyroxine closely in its general physical properties and solubilities; it gave with intensity the colour reaction with nitrous acid and ammonia which is given by thyroxine and many other *o*-diiodophenols.

SUMMARY

1. The possibility is discussed of controlling thyroid function by means of compounds inhibiting the output of thyrotrophic hormone from the anterior pituitary gland.

2. It is pointed out that such compounds may occur among chemical analogues of thyroxine. The synthesis is described of 3:5-diiodo-4(4'-hydroxy)-phenylsulphidophenylalanine, a sulphur-containing analogue of thyroxine, which is designed for the test of this hypothesis.

After this synthesis had been planned and begun I learnt by chance that Dr Paul Block, junr., had done some preliminary experiments on similar lines which circumstances had compelled him to interrupt. Since Dr Block did not have the particular theoretical interest in the work which is indicated by the introduction to this paper, and since the early resumption of his experiments was impossible, it was agreed that the synthesis should be completed in my laboratory. I wish to express my appreciation of Dr Block's attitude in this matter, and also of his generosity in giving me valuable information about the most favourable conditions for carrying out the first reaction.

I am indebted to Dr W. L. M. Perry for carrying out the toxicity tests and to Dr A. S. Parkes for the tests on tadpoles. My thanks are also due to Mr W. Hickford for technical assistance.

REFERENCES

- Gaddum, J. H. (1929–30). *J. Physiol.* **68**, 383.
 Harington, C. R. (1944). *Proc. Roy. Soc. B*, **132**, 223.
 Harington, C. R. & Barger, G. (1927). *Biochem. J.* **21**, 169.
 Harington, C. R. & McCartney, W. (1927). *Biochem. J.* **21**, 852.
 Lamb, J. & Robson, W. (1931). *Biochem. J.* **25**, 1231.
 Lerman, J. & Harington, C. R. (1948). *J. clin. Invest.* **27**, 546.
 Schuegraf, K. (1929). *Helv. chim. Acta*, **12**, 405.
 Suter, C. M. & Hanson, H. L. (1932). *J. Amer. chem. Soc.* **54**, 4100.
 Taurog, A. & Chaikoff, I. L. (1947). *J. biol. Chem.* **171**, 439.