XXIII. CHEMISTRY OF THYROXINE.
III. CONSTITUTION AND SYNTHESIS OF THYROXINE.

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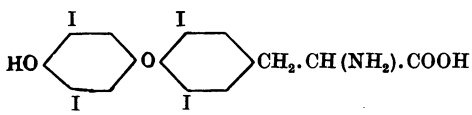
In recently published work it has been shown by one of us [Harington, 1926] that thyroxine is a tetraiodo-derivative of the *p*-hydroxyphenyl ether of tyrosine[1]; the orientation of the iodine atoms was left undetermined but it was suggested that they probably occupied the positions shown in the following formula:



The object of the present paper is in the first place to offer evidence in support of this suggestion, and in the second place to describe the synthesis of thyroxine itself[2].

The experimental and theoretical considerations leading to the above-mentioned supposition regarding the position of the iodine atoms were as follows.

(1) Thyroxine was fused with potash at a high temperature in the absence of oxygen, in the hope of obtaining identifiable degradation products in which iodine was replaced by hydroxyl. Although no pure substances could be isolated as the result of these experiments, the products of alkali fusion did, nevertheless, exhibit pyrogallol reactions, from which it appeared probable that one or both of the benzene rings of the thyroxine molecule had been converted into 3:4:5-trihydroxy-derivatives.

(2) Kendall and Osterberg [1919] have described a colour reaction with nitrous acid and ammonia as being characteristic of thyroxine; we have found that this colour reaction is given in general by benzene derivatives which contain two iodine atoms in the *ortho* positions to a hydroxyl (or amino-)

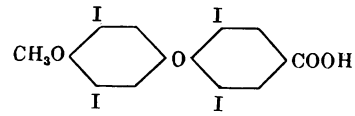
[1] I have since learned that Dr H. D. Dakin had come simultaneously to substantially the same conclusions as myself regarding the constitution of thyroxine; he had made the additional interesting observation that, on heating thyroxine with fuming hydriodic acid at 140°, tyrosine is formed. On hearing from Prof. Barger that I had communicated a paper on the subject to this *Journal*, Dr Dakin withdrew his paper, which was at that time in the hands of the Editor of the *Journal of Biological Chemistry*, from publication. C. R. H.

[2] The claim of Kendall and Osterberg [1919] to have synthesised thyroxine cannot be maintained in view of the facts that no experimental evidence has been offered in support of this claim, and, further, that the views of these authors regarding the constitution of thyroxine have been shown [Harington, 1926] to be wholly erroneous.

group; it is, for instance, given with intensity by diiodo-*p*-cresol, by 3:5-diiodo-4-hydroxybenzoic acid, and by 3:5-diiodotyrosine; this therefore made it likely that thyroxine contained a similar grouping.

(3) On general grounds one is almost bound to regard thyroxine as being derived from tyrosine, through the stage of 3:5-diiodotyrosine; assuming this to be the case, it is highly probable that thyroxine is formed in nature by the coupling of two molecules of diiodotyrosine with the loss of one side chain; such a reaction would lead to a compound of the suggested constitution.

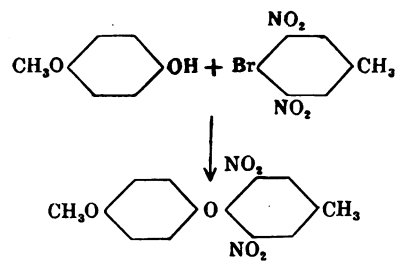
Admittedly these arguments cannot be regarded as convincing, but further evidence by methods of degradation appeared to be out of the question, and the solution of the problem could therefore only be obtained by synthesis. A certain simplification could indeed be effected by the methylation and subsequent oxidation of thyroxine, by a series of steps precisely similar to that described in the case of desiodothyroxine [Harington, 1926] leading to a tetraiodo-derivative of the acid $CH_3O.C_6H_4.O.C_6H_4.COOH$, and our first object was therefore the synthesis of the compound



and its comparison with the corresponding acid derived from thyroxine.

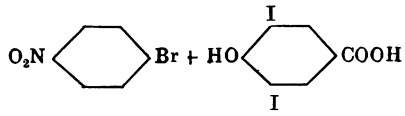
Early experiments on the direct iodination of desiodothyroxine itself and of other *p*-hydroxyphenyl ethers had indicated that these compounds would take up readily two atoms of iodine (though the products were very difficult to purify), but that the uptake of iodine ceased definitely at this point; no method was found for the direct introduction of more than two atoms of iodine. This being so, it was apparent that the desired tetra-iodophenyl ethers could only be obtained by the introduction of those two iodine atoms (or other groups which could be substituted by iodine) which were destined to occupy the 3:5 positons, *before* the phenyl ether synthesis was carried out. In other words, iodine atoms (or other replaceable groups) had to be present in the *ortho* positions either to the halogen atom or to the phenolic group which was to take part in the phenyl ether condensation.

In the first experiments in this direction quinol monomethyl ether was condensed with 3:5-dinitro-4-bromotoluene:



the resulting dinitrodiphenyl ether was successfully reduced to the diamine, but complete failure attended all attempts to replace the amino-groups in this compound by iodine.

This line of attack was therefore abandoned in favour of attempts to condense *p*-nitrohalogenbenzenes with 3:5-diiodo-4-hydroxybenzene derivatives, *e.g.* to condense *p*-nitrobromobenzene with 3:5-diiodo-4-hydroxybenzoic acid:

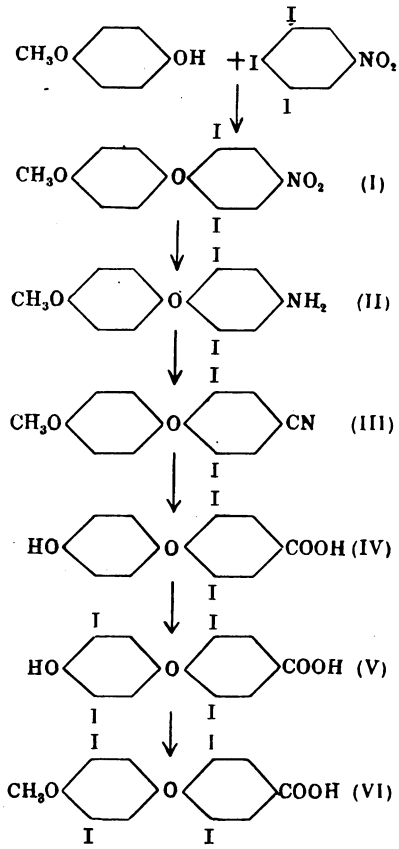


Much time was spent without success on experiments of this type. Mild treatment, such as warming the components in pyridine solution, or boiling in acetone with potassium carbonate, resulted only in the recovery of unchanged starting material, whilst more vigorous treatment, with the addition of copper bronze, caused the elimination of the relatively labile iodine atoms.

The solution of the difficulty was finally obtained in a different manner. As is well known, the presence of a nitro-group exerts a powerful mobilising effect on a halogen in the *para* position, a somewhat less marked effect on one in the *ortho* position, and no effect at all on one in the *meta* position. In accordance with this fact it was found that, of the three iodine atoms in 3:4:5-triiodonitrobenzene, that in the 4-position is so far preferentially mobilised that this substance can be condensed with a phenol to give a good yield of the 3:5-diiodo-4-phenoxynitrobenzene. Quinol monomethyl ether, therefore, was condensed with 3:4:5-triiodonitrobenzene to give 3:5-diiodo-4-(4′-methoxyphenoxy)nitro-benzene (I); this compound was reduced to the corresponding aniline (II) and the latter converted, by means of Sandmeyer's reaction, into the nitrile (III). This nitrile, on boiling with a mixture of hydriodic and acetic acids, underwent simultaneous hydrolysis and demethylation, yielding 3:5-diiodo-4-(4′-hydroxyphenoxy)benzoic acid (IV); on addition of iodine in potassium iodide to a solution of this acid in concentrated ammonia, iodine was rapidly taken up, the uptake ceasing abruptly at two molecules, and there was obtained a good yield of 3:5-diiodo-4′-(3′:5′-diiodo-4′-hydroxyphenoxy)benzoic acid (V); on methylation, this gave an acid (VI) which was in every respect identical with the acid obtained by the methylation and subsequent oxidation of thyroxine. This series of experiments, therefore, settled beyond doubt the question of the orientation of the iodine atoms in thyroxine, since it is certain that the last two iodine atoms, introduced into the acid (IV) in alkaline solution, must have entered the *ortho* positions to the free phenolic group.

As regards the actual synthesis of thyroxine, the most favourable starting point appeared to be 3:5-diiodo-4-(4′-methoxyphenoxy)benzaldehyde (VII) which could indeed be obtained in good yield from the nitrile (III) by the method of Stephen [1925]. It was obvious, however, that formidable diffi-

culties were to be expected, since the reduction which would be involved in the synthesis, from this aldehyde, of the corresponding α-aminopropionic acid would be likely to displace the iodine atoms; indeed, for this reason, an alkaline reduction was entirely out of the question, and prolonged heating with hydriodic acid and red phosphorus proved to be equally unsuccessful. The first success was obtained by condensing the aldehyde with hydantoin, boiling the product for one hour with hydriodic acid and a little phosphorus,



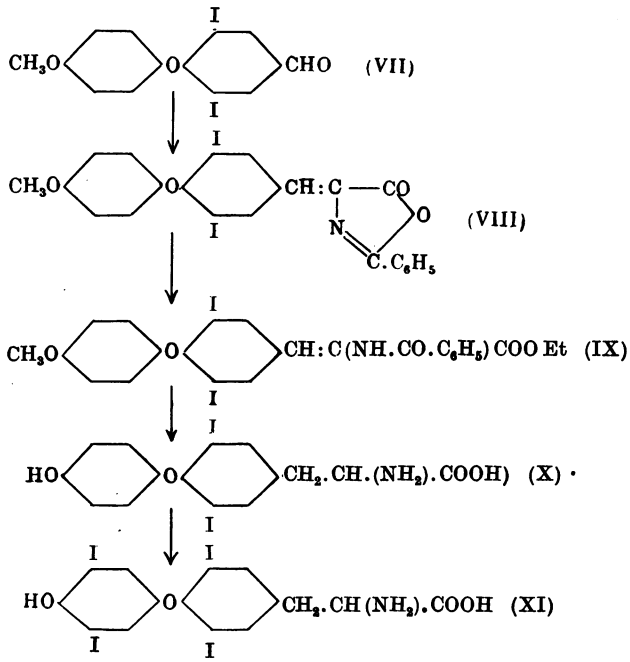
and hydrolysing the resulting compound with concentrated barium hydroxide; the intermediate products were, however, very insoluble and difficult to purify, and the yields were poor. Considerably better results were obtained as follows: the aldehyde (VII) was condensed with hippuric acid, and the resulting azlactone (VIII) converted into the corresponding α-benzoylamino-cinnamic ester (IX); this, on boiling for one hour with hydriodic acid and red phosphorus, gave a 25 % yield of

β-[3:5-diiodo-4-(4′-hydroxyphenoxy)]phenyl-α-aminopropionic acid (X).

On iodination in ammoniacal solution this compound yielded

β-[3:5-diiodo-4-(3′:5′-diiodo-4′-hydroxyphenoxy)]phenyl-α-aminopropionic acid (XI),

a substance which was entirely identical, in its chemical and physiological properties, with thyroxine isolated from the thyroid gland. By this synthesis, therefore, together with the confirmatory evidence offered by the synthesis of the iodine-containing degradation product described above, the constitution of thyroxine may be regarded as finally established.



Experimental.

A. *Degradation of thyroxine.*

Potash fusion of thyroxine. 0·5 g. thyroxine was mixed with 5 g. powdered potassium hydroxide and the mixture heated at 310° for 30 minutes in a silver tube through which a current of hydrogen was passing. After cooling, the melt was dissolved out with hydrochloric acid containing some sulphur dioxide. The product was not entirely soluble, a grey precipitate being obtained, which, as also the solution itself, darkened on exposure to air. On addition of excess of alkali the solution became immediately black. When the solution was carefully neutralised with ammonia and treated with dilute ferric chloride, colour reactions of the pyrogallol type were obtained. Variations of the duration and temperature of the fusion were tried, but in no case could any pure product be isolated.

Exhaustive methylation of thyroxine. 2·33 g. thyroxine were dissolved in 55 cc. methyl alcohol containing 1 % potassium hydroxide; 25 cc. of methyl iodide were added and the solution boiled under a reflux condenser; boiling was continued for 8 hours in all, additions of 10 cc. of 4 % methyl alcoholic

potassium hydroxide being made at half-hourly intervals, and two further quantities of 5 cc. of methyl iodide being added at 4 and 6 hours respectively; 100 cc. of water were then added and the methyl alcohol and excess of methyl iodide distilled off under reduced pressure; the colourless product which separated was filtered off and boiled with 50 cc. alcohol and 50 cc. 2*N* potassium hydroxide until trimethylamine ceased to be evolved; the hot clear solution, on cooling, deposited 1·6 g. of the potassium salt of the unsaturated acid. The free acid, on crystallisation from glacial acetic acid, in which it was but little soluble, formed fine colourless needles which gave off iodine at 286° and melted indefinitely above 290°.

Analysis. 5·747 mg. gave 5·340 mg. CO_2; 0·80 mg. H_2O.
1·272 mg. required 7·73 cc. *N*/196 thiosulphate [Kendall, 1914].

	C	H	I
Found	25·3 %	1·5 %	65·75 %
Calc. for $C_{16}H_{10}O_4I_4$	24·8 %	1·3 %	65·5 %

Oxidation of unsaturated acid, $C_{16}H_{10}O_4I_4$. 0·81 g. of the potassium salt of the above acid was suspended in 35 cc. boiling water, and treated gradually with 1·5 *N* potassium permanganate; after the latter had been added in amount corresponding to 3 atoms of oxygen the oxidation ceased abruptly; apparently the aldehyde is so completely insoluble in water as to escape further oxidation under these conditions. The solution was treated with sulphur dioxide, and the amorphous precipitate, consisting entirely of neutral material, was filtered off, washed, dried and crystallised from glacial acetic acid; it formed sheaves and rosettes of fine colourless needles, M.P. 198°.

Analysis. 4·515 mg. gave 3·890 mg. CO_2; 0·51 mg. H_2O.
1·37 mg. gave 0·9489 mg. I [Kendall, 1914].

	C	H	I
Found	23·5 %	1·3 %	69·3 %
Calc. for $C_{14}H_8O_3I_4$	23·0 %	1·1 %	69·5 %

Oxidation of aldehyde, $C_{14}H_8O_3I_4$. 0·2 g. of the above aldehyde was dissolved in 4 cc. pure pyridine and the solution treated gradually, in the cold, with 0·6 cc. of 5 % potassium permanganate; after the latter had been entirely decolorised, the solution was diluted with water, treated with sulphur dioxide until colourless, made acid to Congo red with hydrochloric acid, and the white precipitate filtered off. The product was entirely soluble in a large amount of boiling dilute sodium carbonate, but, on cooling, the sodium salt separated almost completely in well-formed colourless needles. The free acid, crystallised from glacial acetic acid, formed a felt of fine colourless needles, M.P. 283°.

Analysis. 4·443 mg. gave 3·720 mg. CO_2; 0·53 mg. H_2O.
1·466 mg. gave 1·0019 mg. I [Kendall, 1914].

	C	H	I
Found	22·8 %	1·3 %	68·3 %
Calc. for $C_{14}H_8O_4I_4$	22·5 %	1·1 %	67·9 %

The *methyl ester*, prepared in the usual way, and recrystallised from acetic acid, formed flattened rhombic prisms, M.P. 233°; the *ethyl ester* formed long thin needles, M.P. 171·5°.

B. *Synthesis of acid degradation product,* $C_{14}H_8O_4I_4$ (VI).

3:4:5-Triiodonitrobenzene was most conveniently prepared from diiodo-*p*-nitraniline (obtained by the method of Willgerodt and Arnold [1901]) as follows: 75 g. diiodo-*p*-nitraniline were dissolved in 150 cc. concentrated sulphuric acid; the solution was cooled to 5° and vigorously stirred while finely powdered sodium nitrite (2·5 mols.) was slowly added; the stirring was continued for a further 2 hours, the temperature being maintained at 5–10° throughout. The mixture was then poured on to ice and treated with a concentrated aqueous solution of potassium iodide (5 mols.). After the first vigorous reaction, the solution was warmed for some time on the water-bath; it was then cooled, free iodine was removed with bisulphite, and the granular precipitate filtered off, washed with water, alcohol and ether, and dried. The most convenient purification was effected by distillation *in vacuo*, which gave a clean product, M.P. 165°, in a yield of 75–80 % of the theoretical. The M.P. of this compound is given by Willgerodt and Arnold [1901] as 105°; in a later paper by Repossi [1916] the figure given is 167°[1].

3:5-*Diiodo*-4-(4′-*methoxyphenoxy*)*nitrobenzene* (I). This compound was prepared by boiling a solution of quinol monomethyl ether and 3:4:5-triiodonitrobenzene in methylethylketone with dry potassium carbonate. If molecular proportions were employed, the product contained a good deal of unchanged triiodonitrobenzene which is difficult to remove; it was found better, therefore, to use twice the theoretical amount of the phenol and potassium carbonate. It is further essential for the success of this reaction that the potassium carbonate should be freshly dried and finely powdered. 50 g. 3:4:5-triiodonitrobenzene and 25 g. quinol monomethyl ether were dissolved in 250 cc. boiling methylethylketone; 30 g. finely powdered anhydrous potassium carbonate were added and the solution boiled under a reflux condenser for 16 hours; it was then poured into water containing a slight excess of acetic acid, and the methylethylketone and excess of quinol monomethyl ether were removed by steam distillation; after cooling, the precipitate was filtered off, and crystallised, first from glacial acetic acid, and then from methylethylketone; there resulted 33–35 g. of a product which was practically pure, *i.e.* a yield of about 67 %. For complete purification the compound was distilled *in vacuo* (B.P. 260°/3 mm.) and crystallised from methylethylketone. It formed yellow elongated prisms, M.P. 144°. It is insoluble in water, slightly soluble in alcohol and ether, much more readily so in chloroform and acetic acid.

Analysis. 0·2257 g. gave 0·2593 g. CO_2; 0·0474 g. H_2O.
0·1581 g. gave 0·1502 g. AgI.
20·1 mg. gave 0·523 mg. N (modified micro-Kjeldahl).

	C	H	N	I
Found	31·3 %	2·3 %	2·6 %	51·3 %
Calc. for $C_{13}H_9O_4NI_2$	31·2 %	1·8 %	2·8 %	51·1 %

[1] This compound has also been recently prepared in a similar manner by Kalb, Schmeizer, Zellner and Berthold (*Ber. deutsch. chem. Ges.* 1926, **59**, 1860. [C. H. R. February 2, 1927.]

3:5-*Diiodo*-4-(4′-*methoxyphenoxy*)*aniline* (II). 25 g. of the above nitro-compound were dissolved in 125 cc. hot glacial acetic acid; 37·5 g. powdered stannous chloride were added, and a rapid stream of dry hydrogen chloride passed into the solution, which was kept hot on the water-bath. After a time the stannichloride of the base began to separate; when the precipitate no longer increased in amount and the solution was saturated with hydrochloric acid (about 45 minutes), it was allowed to cool; the crystalline stannichloride was then filtered off, washed with glacial acetic acid, decomposed as rapidly as possible by grinding with 250 cc. of 40 % sodium hydroxide, and the base immediately extracted with ether. If the operation had been properly conducted, the ethereal solution was but little coloured, in which case it was used, after drying, for the direct preparation of the hydrochloride of the base which was required for the next reaction. The free base was obtained pure by evaporating the dried ethereal solution and recrystallising the residue, first from a very small amount of benzene, and then from ligroin. It formed colourless narrow rhombic prisms, M.P. 121–122°. It was very slightly soluble in hot water, but easily soluble in most organic solvents except light petroleum.

Analysis. 0·1782 g. gave 0·2204 g. CO_2; 0·0426 g. H_2O.
0·1629 g. gave 0·1634 g. AgI.
34·2 mg. gave 1·024 mg. N (micro-Kjeldahl).

	C	H	N	I
Found	33·7 %	2·6 %	2·7 %	54·2 %
Calc. for $C_{13}H_{11}O_2NI_2$	33·4 %	2·4 %	3·0 %	54·4 %

The *hydrochloride* formed colourless needles, sparingly soluble in water, M.P. 216° after preliminary sintering; the *sulphate* formed colourless needles, with similar solubilities, M.P. 201° after sintering. The base and its salts have a distinctly irritating effect on the skin, which necessitates considerable care in handling them.

3:5-*Diiodo*-4-(4′-*methoxyphenoxy*)*benzonitrile* (III). The diazotisation of the base just described offered, at first, considerable difficulties, since, in the presence of excess of mineral acid (especially of sulphuric acid) complex coloured condensation products were readily formed. The reaction can however be conveniently accomplished by treatment of a suspension of the dry hydrochloride in glacial acetic acid with amyl nitrite. 30 g. of the hydrochloride, prepared by saturating the ethereal solution of the base with dry hydrogen chloride, were suspended in 300 cc. of glacial acetic acid; the suspension was vigorously stirred and kept at 15–20° while amyl nitrite was gradually added in slight excess; the hydrochloride rapidly disappeared and there was obtained a clear orange-coloured solution of the diazonium salt. For the preparation of the nitrile, this solution was poured, with stirring, into a hot solution prepared by adding 170 g. potassium cyanide in 300 cc. water to 150 g. copper sulphate in 600 cc. water. (It was found to be necessary to use the very large excess of cuprous cyanide here indicated, since otherwise the diazonium group was very readily exchanged for hydrogen.) The precipitate was filtered off, washed with water, and boiled out with benzene; the

benzene solution was filtered and dried over calcium chloride. The benzene was then evaporated and the residue distilled under reduced pressure; the dark-coloured, but crystalline, distillate was recrystallised from methylethylketone, and 13–15 g. of a substance forming colourless prisms, M.P. 167–169°, were obtained.

Analysis. 0·2145 g. gave 0·2760 g. CO_2; 0·0382 g. H_2O.
0·1650 g. gave 0·1625 g. AgI.
20·0 mg. gave 0·574 mg. N (micro-Kjeldahl).

	C	H	N	I
Found	35·1 %	2·0 %	2·9 %	53·2 %
Calc. for $C_{14}H_9O_2NI_2$	35·2 %	1·9 %	2·9 %	53·2 %

3:5-*Diiodo*-4-(4′-*hydroxyphenoxy*)*benzoic acid* (IV). 5 g. of the nitrile were dissolved in 40 cc. glacial acetic acid, and to the boiling solution, contained in a flask under a reflux condenser, were slowly added 50 cc. hydriodic acid (Sp. G. 1·7) so as to cause as little precipitation of the nitrile as possible; the whole was then boiled for 2 hours. On cooling and diluting with water a partly crystalline precipitate separated, which was filtered off and dissolved in warm dilute ammonia; the filtered ammoniacal solution was acidified and the acid filtered off, dried and crystallised from 50 % alcohol; it formed long colourless needles, M.P. 252–254°. The yield was 80 % of the theoretical.

Analysis. 0·1683 g. gave 0·2004 g. CO_2; 0·0275 g. H_2O.
0·1626 g. gave 0·1592 g.AgI.

	C	H	I
Found	32·5 %	1·8 %	52·8 %
Calc. for $C_{13}H_8O_4I_2$	32·3 %	1·7 %	52·7 %

During the above-described hydrolysis there was always formed a certain amount of a compound containing no nitrogen, and exhibiting phenolic but no acidic properties; this was not investigated further.

3:5-*Diiodo*-4-(3′:5′-*diiodo*-4′-*hydroxyphenoxy*)*benzoic acid* (V). Attempts to iodinate the diiodo-acid, by the usual method for phenols, in solution in sodium or potassium hydroxide, met with no good result; the reaction proceeded smoothly, however, in ammoniacal solution by the method of Datta and Prosad [1917]. 0·964 g. of the diiodo-acid was dissolved in 8 cc. of concentrated ammonium hydroxide (Sp. G. 0·880); to this solution was added, drop by drop, with constant shaking, a normal solution of iodine in potassium iodide; as each drop was added the black precipitate of nitrogen iodide disappeared instantaneously, on shaking, until almost the theoretical 2 mols. of iodine had been added; with further addition of iodine, the black precipitate disappeared more slowly, until, when the theoretical amount (or one or two drops in excess) had been added, the dark colour became permanent, indicating the presence of nitrogen iodide in excess; the end-point of the reaction was almost as sharply defined as that of a titration. After about 75 % of the iodine had been added a crystalline ammonium salt began to separate and increased in amount until the end of the reaction; addition of saturated ammonium chloride solution precipitated still more of this salt, the total amount of which was 93 % of the theoretical for the ammonium salt of the

tetra-iodo-acid. The free acid, crystallised from glacial acetic acid, formed bunches of colourless needles, M.P. 255°, with decomposition and evolution of iodine.

Analysis. 0·1045 g. gave 0·1325 g. AgI.

Found I 68·5 % Calc. for $C_{13}H_6O_4I_4$ I 69·2 %

3:5-*Diiodo*-4-(3′:5′-*diiodo*-4′-*methoxyphenoxy*)*benzoic acid* (VI). On methylation with dimethyl sulphate and potassium hydroxide in the usual manner, the above-described tetra-iodohydroxy-acid yielded the methoxy-acid, which, when crystallised from glacial acetic acid, formed a felt of colourless needles, M.P. 286°.

Analysis. 1·31 mg. required 8·4 cc. *N*/200 sodium thiosulphate [Kendall, 1914].

Found I 68·0 % Calc. for $C_{14}H_8O_4I_4$ I 67·9 %

The *sodium salt* of the acid was very sparingly soluble in water and crystallised in fine needles; the *methyl ester*, crystallised from acetic acid, formed flattened rhombic prisms, M.P. 233·5°; the *ethyl ester* similarly gave long fine needles, M.P. 172·5°. The results of comparison of this acid and its derivatives with the corresponding compounds obtained, as described above, by degradation of thyroxine are summarised in Table I; they leave no doubt as to the identity of the synthetic acid with that obtained from natural sources.

Table I.

	Crystalline form	M.P.	Mixed M.P.
Synthetic acid	Felted needles	286°	283–4°
Natural acid	,,	283°	
Synthetic methyl ester	Flattened rhombic prisms	233·5°	233°
Natural ,,	,, ,,	233°	
Synthetic ethyl ester	Long needles	172·5°	172·5°
Natural ,,	,,	171·5°	

C. *Synthesis of thyroxine.*

3:5-*Diiodo*-4-(4′-*methoxyphenoxy*)*benzaldehyde* (VII). Our first attempts to prepare this aldehyde from the corresponding nitrile, using the conditions recommended by Stephen [1925], *i.e.* 1·5 mols. of stannous chloride and a period of 2 hours' standing, resulted in minute yields only. Much better results were obtained by increasing the proportion of stannous chloride to 4 mols., and allowing the mixture to stand overnight, or even longer. 5 g. of 3:5-diiodo-4-(4′-methoxyphenoxy)benzonitrile (see above) were dissolved in 35 cc. chloroform and added, with vigorous shaking, to a solution of 12 g. anhydrous stannous chloride in 60 cc. dry ether saturated with hydrogen chloride; after standing for at least 16 hours the yellow precipitate was filtered off,washed with ether and warmed with water containing a little hydrochloric acid; at the boiling point the hydrolysis was almost instantaneous, the yellow stannichloride being replaced by a colourless flocculent precipitate of aldehyde; this was filtered off, washed with dilute hydrochloric acid and then with water, dried, and crystallised from glacial acetic acid; it formed colourless prisms, M.P. 121°. The yield varied from 70 % to 100 % of the theoretical. The

aldehyde was practically insoluble in water, but fairly soluble in all organic solvents except light petroleum.

Analysis. 0·1643 g. gave 0·2131 g. CO_2; 0·0324 g. H_2O.
0·1479 g. gave 0·1447 g. AgI.

	C	H	I
Found	35·3 %	2·2 %	52·9 %
Calc. for $C_{14}H_{10}O_3I_2$	35·0 %	2·1 %	52·9 %

The *phenylhydrazone* formed long yellow needles, M.P. 175–176°.

Azlactone from above aldehyde and hippuric acid (VIII). An intimate mixture of 5 g. of the aldehyde, 5 g. freshly fused sodium acetate, and 1·9 g. hippuric acid was treated with 15 cc. acetic anhydride and heated on the water-bath for 15 minutes; the yellow reaction mixture was ground up with water, filtered off, well washed and dried; the yield was 6 g. of an almost pure product. Crystallised from glacial acetic acid, it formed long, bright yellow needles, M.P. 211°.

Analysis. 15·5 mg. gave 0·303 mg. N (micro-Kjeldahl).
0·1539 g. gave 0·1174 g. AgI.

	N	I
Found	2·0 %	41·2 %
Calc. for $C_{23}H_{15}O_4NI_2$	2·2 %	40·9 %

α-Benzoylamino-3:5-diiodo-4-(4′-methoxyphenoxy)cinnamic ester (IX). The azlactone was warmed on the water-bath for 45–60 minutes with 75 parts of absolute alcohol containing 10 % sulphuric acid; the clear and almost colourless solution was poured into water and the precipitate collected, washed and dried. The yield was practically quantitative; the compound crystallised from glacial acetic acid, or from methylethylketone, in colourless needles, which softened at 195° and melted at 203°.

Analysis. 13·9 mg. gave 0·267 mg. N (micro-Kjeldahl).
0·1482 g. gave 0·1051 g. AgI.

	N	I
Found	1·9 %	38·3 %
Calc. for $C_{25}H_{21}O_5NI_2$	2·1 %	38·0 %

β-[3:5-Diiodo-4-(4′-hydroxyphenoxy)phenyl]-α-aminopropionic acid (X). 5 g. of the benzoylaminocinnamic ester were boiled under a reflux for one hour with 25 cc. hydriodic acid (Sp. G. 1·7) and 3 g. red phosphorus; the solution was filtered hot through asbestos and evaporated to dryness *in vacuo*; some water was added and the evaporation repeated; the residue was dissolved in warm water with the aid of a little hydrochloric acid, and filtered from some tar. In order to avoid separation of the somewhat insoluble hydrochloride of the amino-acid it was necessary not to cool the acid filtrate more than could be helped; as soon as it was cool enough it was shaken out once with ether, which removed the whole of the coloured impurities; the aqueous solution was then brought to the boil and treated with saturated sodium acetate until no longer acid to Congo red; the amino-acid immediately separated in beautiful silvery platelets, which, after cooling, were filtered off, washed with water, alcohol and ether, and dried. The yield was 1 g. of the

pure substance, or 25 % of the theoretical. The compound gave the ninhydrin reaction, but was too insoluble to permit of a satisfactory determination of amino-nitrogen by the Van Slyke method. It melted at 245–6°.

Analysis. 18·1 mg. gave 0·475 mg. N (micro-Kjeldahl).
0·1270 g. gave 0·1118 g. AgI.

	N	I
Found	2·6 %	47·6 %
Calc. for $C_{15}H_{13}O_4NI_2$	2·7 %	48·3 %

β-[3:5-Diiodo-4-(3′:5′-diiodo-4′-hydroxyphenoxy)phenyl]-α-aminopropionic acid—Thyroxine (XI). 0·525 g. of the diiodoamino-acid was dissolved in 11–12 cc. concentrated ammonium hydroxide (Sp. G. 0·880) and treated, drop by drop, with constant shaking, with *N* iodine in potassium iodide (2 mols.); towards the end of the reaction, the uptake of iodine, which until then had been practically instantaneous, showed a distinct slackening; during the later stages of the reaction a crystalline ammonium salt had begun to separate; after all the iodine had been added, the solution was allowed to stand for some time, and the ammonium salt was then separated on the centrifuge; the salt was dissolved in alcohol with the aid of a little dilute sodium hydroxide, the solution was filtered, boiled and acidified with acetic acid; there separated immediately a colourless crystalline precipitate, which exhibited the very characteristic crystalline form of thyroxine (rosettes and curiously shaped sheaves of colourless needles). This fraction amounted to 0·33 g. A further amount was obtained from the mother-liquor of the ammonium salt as follows: the solution was diluted and boiled until the ammonia was removed; the partly crystalline precipitate which had separated was filtered off and dissolved, so far as possible, in boiling 0·5 % sodium carbonate; the filtered solution, on cooling, deposited a heavy white micro-crystalline precipitate, with the typical appearance of the sodium salt of thyroxine; this was separated on the centrifuge and further treated as described above for the ammonium salt; in this way 0·07 g. of somewhat less well-defined crystalline material was obtained, the total yield, therefore, being 0·4 g., or a little over 50 % of the theoretical. The substance melted at 231° with decomposition; when mixed with a sample of natural thyroxine of M.P. 228°, the mixture melted at 228°; it gave with intensity the colour reaction with nitrous acid and ammonia which is given by thyroxine; further, the solubilities of the natural and synthetic products and of their sodium salts were entirely similar.

Analysis. 24·6 mg. gave 0·447 mg. N (micro-Kjeldahl).
0·99 mg. required 6·08 cc. *N*/200 sodium thiosulphate [Kendall, 1914].

	N	I
Found	1·82 %	65·1 %
Calc. for $C_{15}H_{11}O_4NI_4$	1·82 %	65·3 %

In so far, then, as the matter is susceptible of decision by chemical methods, the identity of the synthetic product with natural thyroxine may be regarded as established. For the physiological confirmation we are indebted to Prof.

D. Murray Lyon of the University of Edinburgh, a note by whom, on the physiological test, is appended.

We have had the advantage of repeated discussion of the problem with Prof. R. Robinson, F.R.S., and we desire to acknowledge the assistance of Dr C. P. Stewart and of Mr W. McCartney in some of the earliest and latest experiments respectively.

Note on Physiological Test of Synthetic Thyroxine.

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Case 1. Mrs A. S. Age 61. Housewife.

Known to be myxoedematous in 1921, probably longer. Admitted to hospital in October 1922 showing typical picture of that condition. The patient improved for a time under thyroid extract, but extract of anterior lobe of pituitary did not prevent relapse. Thyroid medication was not continued after discharge from hospital (January 1923).

November 24th, 1926. Third admission to hospital. The patient has taken no care of herself and has never had thyroid extract outside of hospital. She now shows the classical signs of the disease, is dull mentally, is overweight, and has the typical facies and myxoedematous deposits and the harsh dry skin.

The B.M.R. was practically the same as on her previous admission. On November 27th 5 mg. synthetic thyroxine was dissolved in a drop of normal NaOH and made up to 1·5 cc. with distilled water, and was given intravenously. Next day the patient complained of headache and palpitation. By November 29th, two days after the first injection, the B.M.R. had risen from − 32 % to − 17 %. On the 30th the patient herself remarked on her improved condition. She was much brighter and more cheerful, her swellings were diminishing, and wrinkling was obvious on the backs of the hands and under the eyes. Two further doses of 4 and 5 mg. given on November 29th and December 2nd raised the B.M.R. to − 6 % by December 4th. During this period both pulse rate and temperature rose somewhat. A fourth dose given on December 6th seems to have had little effect, as B.M.R., pulse rate and temperature have all declined from about this date. By the 18th the patient had practically returned to her previous condition with B.M.R. − 34 %. The skin had become harsh again and the mental condition much more dull.

The close parallelism between B.M.R. and pulse rate will be noticed in Fig. 1.

Case 2. Mrs M. M. Age 35. Housewife.

Normal health until 1916 when she was noticed to be becoming dull mentally and disinclined for effort. Definitely diagnosed myxoedema in 1920. For next few years took thyroid tablets irregularly.

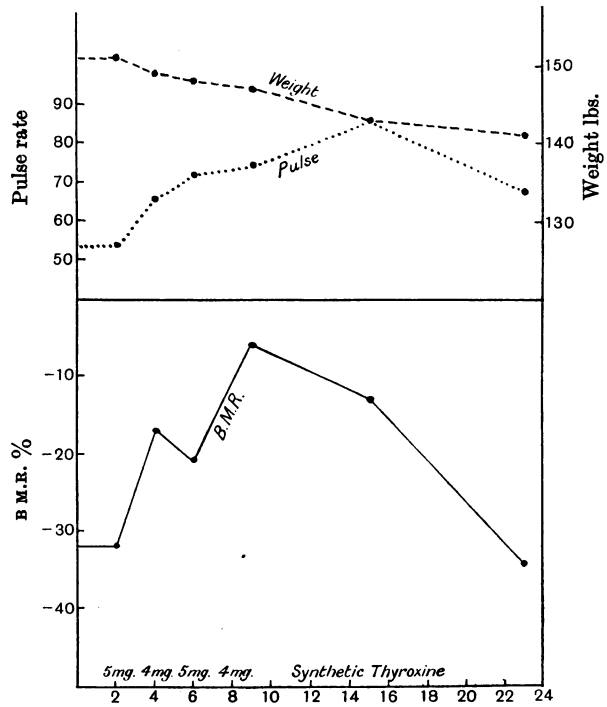

Fig. 1.

January 1926 admitted to Royal Infirmary, Edinburgh, on account of lack of energy and great increase in her swellings. Picture typically that of advanced myxoedema, with a B.M.R. of − 46 %. Remained in hospital from January 5th to March 29th while the dosage of thyroid extract was adjusted. Twelve grains a day proved to be too much and the B.M.R. rose to + 25 %. She was finally discharged on 6 grains a day with a B.M.R. of + 4 %.

Re-admitted November 24th, 1926, not having had any thyroid extract for some months. B.M.R. again − 45 %, the patient being markedly myxoedematous. Three intravenous injections of synthetic thyroxine (5, 4 and 5 mg.) were given on November 27th and 29th, and December 2nd. These produced a marked rise in the B.M.R. to + 3 %, the temperature and the pulse rate rising simultaneously. A fall in weight took place during the same period—partly due to great loss of fluid from the system. A very considerable diuresis followed the first two doses of thyroxine. Each injection also gave rise to an

attack of diarrhoea. As the result of the thyroxine the patient improved remarkably for a time, but after it was discontinued, she slipped back to her former state. In this patient a greater and more lasting effect was produced than in Case 1 (see Fig. 2).

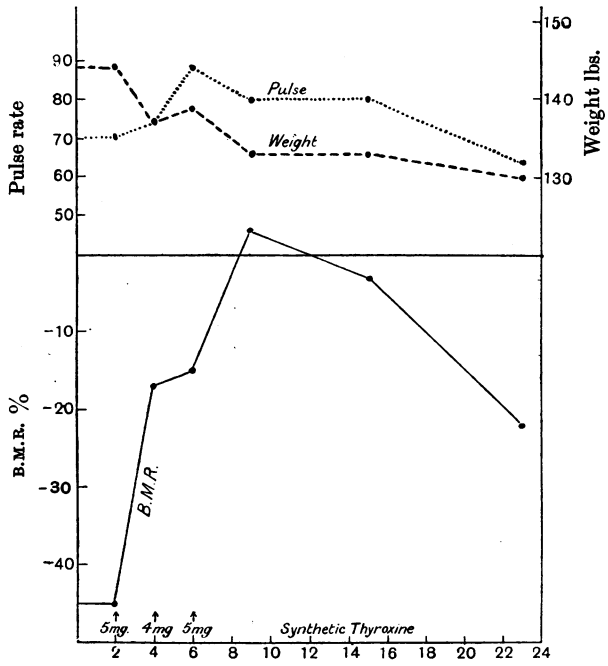

Fig. 2.

The results obtained in the above two cases are shown in Figs. 1 and 2 respectively. The effect of the synthetic thyroxine in raising the basal metabolic rate of these two patients is quantitatively similar to that reported by Boothby and Sandiford [1924] for natural thyroxine.

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