XL. CHEMISTRY OF THYROXINE.

II. CONSTITUTION AND SYNTHESIS OF DESIODO-THYROXINE.

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The method for the isolation of thyroxine described in the preceding paper brought the preparation of comparatively large quantities of the material within the bounds of possibility. For help in the large scale preparation of thyroxine for the purpose of the chemical work here recorded, I am greatly indebted to Messrs The British Drug Houses, Ltd., and in particular to their director, Mr F. H. Carr, under whose supervision about 100 g. of crude crystalline thyroxine have been prepared for me.

In attacking the general problem of the chemical constitution of thyroxine, it was obviously desirable in the first instance to obtain the substance free from iodine, so that the first experiments were directed towards this end. The iodine is readily removed from thyroxine by the usual reducing agents, *e.g.* sodium amalgam or aluminium-mercury couple, but in these cases the reaction products are not easy to obtain pure. A satisfactory solution was found in the use of the palladium hydroxide-calcium carbonate catalyst of Busch and Stöve [1916]; on shaking a 1 % solution of thyroxine in *N* potassium hydroxide in an atmosphere of hydrogen, using the above catalyst, the iodine was rapidly and quantitatively split off as potassium iodide and an almost theoretical yield was obtained of iodine-free thyroxine (to be called hereafter desiodo-thyroxine). This method for the removal of the iodine had the great advantage that, by performing the experiment quantitatively, it was possible to ascertain whether the hydrogen taken up was equivalent to the iodine liberated (*i.e.* 4 mols. per mol. of thyroxine), or whether it amounted to more than this quantity. Such an experiment, recorded in the experimental part below, indicated that the hydrogen taken up was indeed equivalent to the iodine split off, and it is therefore safe to assume that the removal of the iodine had taken place without the induction of secondary reductive changes within the molecule. The desiodo-thyroxine so obtained proved to have the empirical formula $C_{15}H_{15}O_4N$; it gave Millon's reaction and the ninhydrin reaction, and yielded the whole of its nitrogen with nitrous acid in Van Slyke's apparatus; it formed salts with acid and alkali; it was probably, therefore, an α-amino-acid with at least one phenolic group. The proportions of carbon and hydrogen suggest the presence of two benzene rings.

On fusion with potassium hydroxide, the products varied with the temperature. On fusion at 250° in an open vessel, the products were *p*-hydroxybenzoic acid, a substance $C_{13}H_{12}O_2$, and a minute amount of quinol, together with ammonia and oxalic acid; on fusion at 310° in an atmosphere of hydrogen there were obtained *p*-hydroxybenzoic acid and quinol in good yield, and again ammonia and oxalic acid; the substance $C_{13}H_{12}O_2$ possessed apparently only one phenolic group (it gave a monobenzoyl derivative), the second oxygen being inert. These experiments in the first place reinforce the suggestion of the presence of two benzene rings, one at least of which has a phenolic or phenol ether group in the *para*-position to a side chain from which a two carbon fragment is split off as oxalic acid; it is unsafe to draw rigid conclusions as to the origin of the quinol, owing to the possibility of the introduction of an additional phenolic group or the production of isomeric change during the fusion.

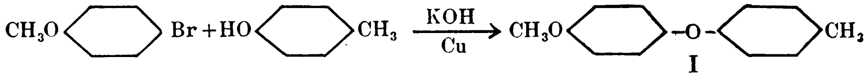
On exhaustive methylation, desiodo-thyroxine behaved similarly to tyrosine in that the completely methylated product (betaine) lost trimethylamine with ease on boiling with alkali, and gave an unsaturated acid, $C_{16}H_{14}O_4$; methoxyl determinations on this acid indicated one methoxyl group. On oxidation with potassium permanganate (30) this acid gave a neutral substance, $C_{14}H_{12}O_3$, together with oxalic acid. The substance $C_{14}H_{12}O_3$ gave a semicarbazone and a phenylhydrazone; since its formation in the above oxidation occurred in good yield and was accompanied by the formation of practically no acidic products, it was at first thought to be a ketone; further investigation showed, however, that the substance obtained by the action of phosphorus pentachloride on its oxime was a nitrile and not the expected anilide; the oxidation product was therefore an aldehyde, and it was then found that on further oxidation with potassium permanganate it yielded an acid, $C_{14}H_{12}O_4$, which was stable to further boiling with alkaline permanganate. Reviewing the results up to the present point then, we have in the first place by the potash fusion demonstrated the probable presence in desiodo-thyroxine of two benzene rings. The behaviour of the compound on exhaustive methylation proves almost with certainty that it is an amino-acid; moreover, the presence of one methoxyl group in the unsaturated acid $C_{16}H_{14}O_4$ proves the presence of one phenolic group only in desiodo-thyroxine; finally, the splitting off of oxalic acid by oxidation, with the formation of a residual stable acid is evidence of the presence of a three carbon side chain; on the experiments hitherto described the degradation may be represented thus:

$$\begin{aligned}\mathrm{HO.C_{12}H_8O.CH_2.CHNH_2.COOH} &\rightarrow \mathrm{CH_3O.C_{12}H_8O.CH:CH.COOH}\\ &\rightarrow \mathrm{CH_3O.C_{12}H_8O.CHO} \rightarrow \mathrm{CH_3O.C_{12}H_8O.COOH}\end{aligned}$$

The remaining question is therefore the character of the two benzene ring group $—C_{12}H_8O—$. The two benzene rings composing this group cannot be linked through a carbon atom, since such a linkage would have led, on the above scheme of degradation, to the formation of a ketone (benzophenone derivative) on oxidation in place of the aldehyde actually obtained. They must therefore be linked either directly (diphenyl) or through the remaining oxygen atom which is as yet unaccounted for. This indifferent oxygen atom would be difficult to account for on the diphenyl hypothesis, so that the existence of an oxide linkage between the two benzene rings seemed the most probable supposition. At this point, therefore, it was decided to attempt to meet the degradation by synthesis, proceeding on the hypothesis that the group —$C_{12}H_8O$— represented diphenyl ether minus two hydrogen atoms.

On condensing *p*-bromoanisol by the method of Ullmann and Stein [1906] with the potassium salt of *p*-cresol in presence of copper bronze there was obtained 4-(4′-methoxyphenoxy) toluene (I, p. 303); this compound, on boiling with hydriodic acid, gave 4-(4′-hydroxyphenoxy) toluene (II) which proved to be identical with the substance $C_{13}H_{12}O_2$, which resulted from the mild potash fusion of desiodo-thyroxine. Further, on boiling with permanganate, the 4-(4′-methoxyphenoxy) toluene gave an acid, 4-(4′-methoxyphenoxy) benzoic acid (III), which was identical with the acid $C_{14}H_{12}O_4$ obtained, as described above, as the final oxidation product of desiodo-thyroxine after methylation.

p-Bromoanisol was then condensed similarly with potassium phenate, giving (4′-methoxyphenoxy) benzene (IV); from this, by Gattermann's hydrocyanic acid method, was prepared an aldehyde. This aldehyde was identical with the aldehyde $C_{14}H_{12}O_3$ obtained in the degradation, and was further proved to be 4-(4′-methoxyphenoxy) benzaldehyde (V) by the fact that, on oxidation, it gave an acid identical with the 4-(4′-methoxyphenoxy) benzoic acid (III) obtained as described above by oxidising 4-(4′-methoxyphenoxy) toluene. From this aldehyde it was then possible to synthesise 4-(4′-methoxyphenoxy) cinnamic acid (VI) and also desiodo-thyroxine itself. By Perkin's reaction the unsaturated acid was obtained and identified with the degradation product $C_{16}H_{14}O_4$. Desiodo-thyroxine was synthesised by two methods; (*a*) by the method of Sasaki [1921]: 4-(4′-methoxyphenoxy) benzaldehyde was condensed with glycine anhydride in presence of acetic anhydride and sodium acetate; on boiling the condensation product with hydriodic acid and red phosphorus it underwent simultaneous reduction, hydrolysis and demethylation with formation of β-[4-(4′-hydroxyphenoxy) phenyl]-α-aminopropionic acid (VII); (*b*) by the method of Wheeler and Hoffmann [1911]: 4-(4′-methoxyphenoxy) benzaldehyde was condensed with hydantoin and the condensation product boiled with hydriodic acid and red phosphorus in the same way. In both cases the synthetic amino-acid was identical with the natural desiodo-thyroxine; the proof is therefore complete that desiodo-thyroxine is β-[4-(4′-hydroxyphenoxy) phenyl]-α-aminopropionic acid, or, more simply, the *p*-hydroxyphenyl ether of tyrosine, and, further, that thyroxine is a tetraiodo-substituted derivative of this compound. The orientation of the iodine atoms in thyroxine remains to be finally determined, but experiments, which are as yet incomplete, indicate that the iodine atoms probably occupy the 3, 5, 3′, 5′ positions.



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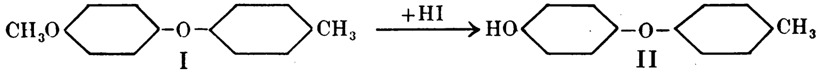

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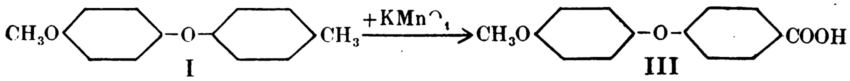

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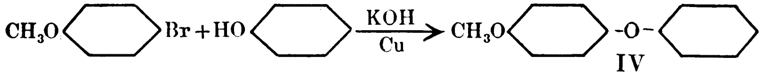

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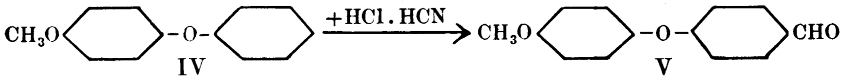

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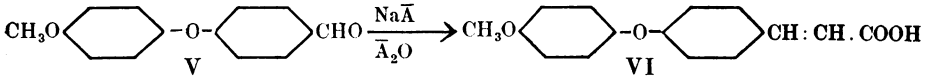

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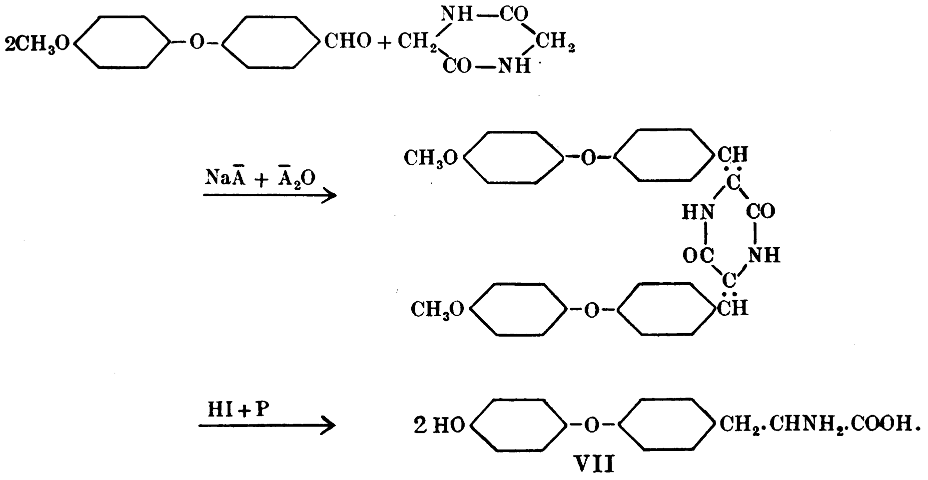

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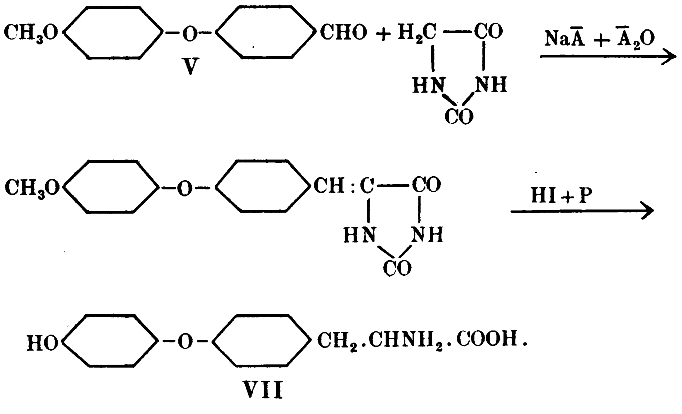

V

VII

EXPERIMENTAL.

A. *Degradation.*

Reduction of thyroxine. 6·0 g. thyroxine were dissolved in 600 cc. of *N* potassium hydroxide. To the solution were added 18 g. of a catalyst prepared by adding 1 g. palladium chloride to a hot aqueous suspension of 50 g. pure calcium carbonate, stirring well, filtering, washing until free from halogen and drying in a desiccator over potash. The solution was then vigorously shaken in an atmosphere of purified hydrogen. After a slight preliminary lag the hydrogen was rapidly absorbed, the end of the reaction being marked by the blackening of the catalyst and a sudden slackening in the uptake of hydrogen. As will be seen from the quantitative experiment recorded below, the uptake of hydrogen corresponded closely to the theoretical 4 mols. At the end of the reaction the catalyst was filtered off and the filtrate acidified with acetic acid; a small flocculent precipitate was removed by filtration and the solution evaporated on the water-bath until crystals began to separate. The solution was then allowed to stand in the cold for some time and the precipitate filtered off; the product so obtained, which was slightly pigmented, but crystalline and almost pure, amounted to 1·85 g. (88 % of the theoretical); it contained no iodine. The product could be recrystallised by dissolving in warm dilute sulphuric acid and treating the hot solution with sodium acetate until no longer acid to Congo red. Even when analytically pure it had a marked tendency to separate in spheres, which showed crystalline structure if it were crystallised rapidly or from concentrated solution. On slow crystallisation from dilute solution it formed bunches of thick needles or prisms. The melting point varied greatly with the rate of heating. On fairly rapid heating the substance melted and decomposed about 253–254°. It formed salts with acids and alkalis, the acid salts being somewhat insoluble in cold water; both acidic and basic salts were soluble in alcohol. The substance itself was almost insoluble in water, alcohol and other organic solvents; it gave a positive ninhydrin reaction, a positive Millon's reaction and a deep orange colour with Pauly's reagent; the glyoxylic reaction was negative; with nitrous acid it gave a deep orange colour, which did not, however, turn pink on adding ammonia as is the case with thyroxine. As will be seen from the analyses below the whole of the nitrogen is in the form of amino-nitrogen. From these observations and from the results obtained on analysis it appeared that the compound was an α-amino-acid with one or two phenolic groups.

Analysis. (*a*) 0·1203 g. gave 0·2893 g. CO_2; 0·0613 g. H_2O.
(*b*) 0·1182 g. gave 0·2850 g. CO_2; 0·0587 g. H_2O.

6·66 mg. gave 0·33 mg. N (Micro-Kjeldahl).
16·65 mg. gave 1·4 cc. moist N at 19° and 764 mm. (Van Slyke).

	C	H	N	NH_2-N
Found	65·6, 65·7	5·65, 5·5	5·0	4·85
Calc. for $C_{15}H_{15}O_4N$	65·9	5·5	5·1	—

The *hydrochloride* was prepared by dissolving reduced thyroxine in hot 1 : 1 hydrochloric acid from which it crystallised on cooling; it showed the same marked tendency to separate in sphaero-crystals. Recrystallised from dilute hydrochloric acid, it formed colourless thin plates, M.P. 237–239° with decomposition.

0·1274 g. gave 0·0587 g. AgCl

Found	Cl	11·4 %
Calc. for $C_{15}H_{16}O_4NCl$	Cl	11·5 %

A quantitative reduction of thyroxine was carried out as follows: 0·150 g. thyroxine was dissolved in 15 cc. *N* potassium hydroxide and treated with 0·5 g. of the palladium catalyst; the bottle containing the solution, after the air had been displaced by hydrogen, was connected with a burette containing hydrogen, the uptake of which could then be measured accurately; after shaking for 75 minutes hydrogen ceased to be absorbed; the amount taken up was 22·2 cc. at 18° and 754 mm. A blank experiment was then immediately run, the same amount of potassium hydroxide and catalyst being shaken for the same period; the uptake of hydrogen was 3·0 cc. The net uptake of hydrogen was therefore 19·2 cc. or 1·576 mg.; 150 mg. thyroxine require 1·545 mg. hydrogen for 4 mols. The uptake was therefore 2 % in excess of the theoretical. After isolation of the reduction product the filtrate and washings from the latter were treated with nitric acid and silver nitrate; the silver iodide amounted to 0·1772 g. corresponding to 0·0958 g. iodine or 97·8 % of the theoretical. It therefore seems justifiable to assume that "desiodo-thyroxine" was thyroxine in which the iodine had been replaced by hydrogen without the induction of any secondary changes.

Potash fusion of desiodo-thyroxine. (1) 1 g. desiodo-thyroxine was finely powdered and stirred into a melt of 5 g. potassium hydroxide and 0·75 cc. water in a nickel crucible which was held in a bath at 250–260°; the fusion was continued until the obvious reaction was over and the melt was becoming thick and pasty (about 45 minutes); during the fusion ammonia was evolved and a slight smell of indole was observed. The melt, after cooling, was dissolved in 100 cc. of water and the clear dark red solution saturated with carbon dioxide. A crystalline precipitate (*a*) separated and, after standing overnight in the ice-chest, was filtered off. The mother liquor was then extracted with ether, which on drying and evaporating left a crystalline residue with a strong phenolic odour (*b*). The aqueous solution was then acidified with hydrochloric acid and again extracted with ether; this ether extract yielded a dark red solid residue (*c*). The aqueous solution contained oxalic acid.

The precipitate (*a*) was sublimed *in vacuo*; the white sublimate had M.P. 56°, on recrystallisation from ligroin it formed large glistening white plates which melted at 72–73° and, on re-heating, at 56°; the substance was, in fact, apparently dimorphic, heating followed by rapid crystallisation giving the low melting point form, and slow crystallisation from a solvent the high melting form. The compound was slightly soluble in hot water, insoluble in cold water and easily soluble in all organic solvents except light petroleum. It gave Millon's reaction.

Analysis. 3·162 mg. gave 8·945 mg. CO_2; 1·80 mg. H_2O.
Found: C, 77·42 %; H, 6·37 %[1].

Molecular weight by Rast's camphor method.
5·1 mg. in 63·5 mg. camphor. Depression of melting point 15·5°.
M.W. = 207.

The analysis and molecular weight determination suited best the formula $C_{13}H_{12}O_2$, which has C 78·0 %, H 6·0 % and M.W. 200. The compound gave, by the Schotten-Baumann method, a *benzoyl* derivative, which, on crystallisation from light petroleum, formed bundles of thick needles, M.P. 75·5–76·5°.

Molecular weight, 5·0 mg. in 47·2 mg. camphor. Depression of melting point, 13·25°.
M.W. = 320.

It was, therefore, a mono-benzoyl derivative and the compound $C_{13}H_{12}O_2$ had probably only one phenolic group.

The second phenolic fraction (*b*) was crystallised from benzene from which it formed long needles, M.P. 167–168°; when mixed with a sample of quinol the M.P. was 168–169°; it was, therefore, almost certainly quinol. The amount was so small as to preclude the possibility of further tests. The phenolic smelling substance in this fraction remained in the benzene and was probably *p*-cresol (see below).

The acidic fraction (*c*) was carefully sublimed under reduced pressure and the sublimate crystallised from water; it formed crystals with the characteristic appearance of *p*-hydroxybenzoic acid. On heating in a test-tube, phenol was given off above the melting point.

19·9 mg. air-dried substance on heating at 100° lost 2·4 mg.

Found	$H_2O = 12{\cdot}06$ %
Calc. for $C_6H_4(OH)COOH, H_2O$	$H_2O = 11{\cdot}54$ %

The anhydrous substance melted at 210°; when mixed with a synthetic sample of *p*-hydroxybenzoic acid of M.P. 209°, the mixture melted at 209·5°. Fraction (*c*) was, therefore, for the most part *p*-hydroxybenzoic acid.

(2) 0·5 g. desiodo-thyroxine was powdered and mixed with 5 g. powdered potassium hydroxide; the mixture was heated for 30 minutes at 310° in a copper tube from which the air had been displaced with hydrogen and through which a current of hydrogen was passed during the fusion and while the melt was cooling. After cooling, the melt was dissolved out with hydrochloric acid containing some sulphur dioxide. There resulted a practically clear and colourless solution. This was thoroughly extracted with ether; the united ethereal extracts were shaken out with dilute sodium carbonate, dried and evaporated, yielding 0·21 g. of a phenolic fraction which crystallised but was contaminated with an oily substance. It was for the most part soluble in water; the aqueous solution was shaken up with light petroleum; the latter gave, on evaporation, a small quantity of an oil which crystallised on seeding with *p*-cresol, but was

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[1] The micro-combustions recorded in this paper were carried out for me by Fein-Chemie, Tübingen, Germany.

too small in amount for complete identification. The greater part of the phenolic fraction was recovered, on concentrating the aqueous solution, in the form of long needles which melted at 169° and gave an unchanged mixed melting point with quinol; on boiling with ferric chloride the smell of quinone was perceptible and crystals of quinhydrone separated on cooling.

The sodium carbonate solution was acidified with hydrochloric acid and extracted with ether yielding 0·16 g. of an acidic fraction, which on crystallisation from water formed crystals with the appearance of *p*-hydroxybenzoic acid.

33·7 mg. air-dried substance lost 4·1 mg. at 100°.

Found	H_2O	12·2 %
Calculated	H_2O	11·54 %

The anhydrous substance melted at 208·5° and, when mixed with a sample of *p*-hydroxybenzoic acid of M.P. 210°, the mixture melted at 210°. Oxalic acid was again detected in the aqueous solution of the products of the fusion. During the fusion under these conditions ammonia was evolved but no smell of indole could be detected.

Exhaustive methylation of desiodo-thyroxine. 1·6 g. desiodo-thyroxine was dissolved in 6 cc. of 2 *N* potassium hydroxide and treated alternately with six additions of 1·2 cc. dimethyl sulphate and 2 cc. of 6·5 *N* potassium hydroxide, with vigorous shaking; at the end of the reaction the solution was slightly diluted with water and treated with potassium hydroxide to make a 2*N* concentration of the latter; it was then boiled under a reflux condenser. Evolution of trimethylamine commenced at once but did not cease for some hours; as the reaction proceeded the potassium salt of the acid which was being formed, together with some potassium sulphate, separated from the solution and was filtered off from time to time. When the evolution of trimethylamine ceased, the solution was cooled and the remaining potassium salt filtered off; the whole of the latter was dissolved in hot water and the filtered solution acidified. The acid which was precipitated was best purified by means of the sodium or potassium salt, both of which were very insoluble in cold water. Finally the acid was crystallised from dilute alcohol or from 50 % acetic acid, yielding colourless glistening leaflets which melted at 175·5°. The acid was very insoluble in water but more soluble in alcohol. It decolorised alkaline potassium permanganate in the cold.

Analysis. 3·430 mg. gave 8·940 mg. CO_2; 1·64 mg. H_2O.

	C	H
Found	71·11 %	5·35 %
Calc. for $C_{16}H_{14}O_4$	71·11 %	5·30 %

2·980 mg. gave 2·46 mg. AgI (Zeisel).

Found	CH_3O	10·91 %
Calc. for one methoxyl group	CH_3O	10·50 %

The *methyl ester*, obtained by saturating the methyl alcoholic solution of the acid with dry hydrogen chloride, formed glistening leaflets from methyl alcohol (in which it was very little soluble in the cold) having M.P. 128·5°.

Analysis. 3·548 mg. gave 9·360 mg. CO_2; 1·82 mg. H_2O.

	C	H
Found	71·97 %	5·74 %
Calc. for $C_{17}H_{16}O_4$	71·83 %	5·64 %

Oxidation of unsaturated acid $C_{16}H_{14}O_4$. 0·35 g. of the acid was dissolved in 7 cc. water with 1 cc. 2*N* sodium hydroxide and the hot solution treated gradually with 8 cc. of 5 % potassium permanganate which was instantaneously reduced; sulphur dioxide was then passed in and the resulting solution was found to contain an oil, which, on cooling and rubbing, crystallised. It was extracted with ether and the ethereal solution shaken with sodium carbonate which removed a minute amount of an acid; the residue obtained on evaporation of the dried ethereal extract was crystallised from light petroleum and formed macroscopic prisms, M.P. 59·5°.

Analysis. 3·706 mg. gave 10·020 mg. CO_2; 1·79 mg. H_2O.

	C	H
Found	73·76 %	5·40 %
Calc. for $C_{14}H_{12}O_3$	73·70 %	5·30 %

It formed a *semicarbazone*, glistening colourless plates, M.P. 210–211°, and a *phenylhydrazone*, long pale yellow needles, M.P. 135–136°. The *oxime* was prepared (since the compound was first thought to be a ketone) by boiling 210 mg. with 70 mg. hydroxylamine hydrochloride and 100 mg. barium carbonate in 2 cc. alcohol for 5 hours; the solution was filtered hot and the residue boiled out with alcohol; the alcohol was evaporated, yielding an oil, which, on the addition of water, immediately crystallised. Recrystallised from light petroleum, it formed colourless leaflets, M.P. 74–75°.

2·354 mg. gave 0·124 cc. N at 25° and 726 mm.

Found	N 5·80 %
Calculated	N 5·76 %

0·3 g. of the oxime was dissolved in 10 cc. of dry ether and treated, with cooling, with 0·5 g. of phosphorus pentachloride in small portions; the resulting deep yellow solution was shaken with ice, the ether layer separated, dried and evaporated. The residue after recrystallisation from alcohol formed colourless rhombic plates, M.P. 107·5°. On hydrolysis by refluxing with strong alcoholic potassium hydroxide, this gave exclusively an acid which had M.P. 176° and was identical with the further oxidation product $C_{14}H_{12}O_4$ (see above). This experiment finally proved the compound $C_{14}H_{12}O_3$ to be an aldehyde, the product of the action of phosphorus pentachloride on the oxime being a nitrile. The aqueous solution after separation of the aldehyde contained oxalic acid; this was isolated in one experiment and amounted to about 50 % of the theoretical (assuming 1 mol. per mol. of the acid $C_{16}H_{14}O_4$).

Acid $C_{14}H_{12}O_4$. On boiling the aldehyde $C_{14}H_{12}O_3$ with potassium permanganate in slight excess of the theoretical amount and, when oxidation was complete, passing sulphur dioxide into the solution, there was obtained an acid, which on recrystallisation from dilute alcohol formed colourless plates, M.P. 177°. This acid was stable to further boiling with permanganate.

3·268 mg. gave 8·240 mg. CO_2; 1·52 mg. H_2O.
3·136 mg. gave 2·88 mg. AgI (Zeisel).

	C	H	CH_3O
Found	68·79 %	5·20 %	12·14 %
Calc. for $CH_3O.C_{12}H_8O.COOH$	68·85 %	4·92 %	12·7 %

B. *Synthesis.*

4-(4′-*Methoxyphenoxy*) *toluene.* A mixture of 18·7 g. *p*-bromoanisol, 13·5 g. *p*-cresol, 5·6 g. potassium hydroxide and 0·1 g. copper bronze in a small flask with an air condenser, was heated slowly to 235° and maintained at that temperature for 3 hours. A vigorous reaction occurs at about 160° and it is well to heat slowly until this is over; the top of the air condenser was bent so that the mixture of bromoanisol and water, which at first distilled, might be collected. This mixture was separated, when the distillation had ceased, and the bromoanisol dried and returned to the reaction flask. At the end of the reaction, a point which was marked by a considerable slackening in the rate of refluxing, the mixture was cooled and shaken up with ether and water to which had been added excess of potassium hydroxide; the ethereal layer, after separation, was once more shaken out with dilute potassium hydroxide, then separated and dried. The residual oil, after removal of the ether, was distilled under reduced pressure; the distillate crystallised on cooling. The yield was 50–60 % of the theoretical. Recrystallised from dilute alcohol the compound formed colourless plates, M.P. 47–48°.

Analysis. 0·1306 g. gave 0·3745 g. CO_2; 0·0765 g. H_2O.

	C	H
Found	78·2 %	6·5 %
Calc. for $C_{14}H_{14}O_2$	78·5 %	6·5 %

4-(4′-*Hydroxyphenoxy*) *toluene.* On boiling the above compound for 2 hours with 10 parts of hydriodic acid (sp.g. 1·7), diluting with water and cooling, a crystalline precipitate separated out; this was extracted with ether, the ether shaken with sodium hydroxide solution and the latter separated and acidified. The precipitate was filtered off, dried and crystallised from light petroleum; it formed colourless leaflets and showed the same two melting points, 56–57° and 72–73° as the compound $C_{13}H_{12}O_2$ from the potash fusion of reduced thyroxine. Further it gave a *benzoyl* derivative which formed bunches of thick needles and prisms from light petroleum, M.P. 75–77°. It was therefore identical with the compound $C_{13}H_{12}O_2$.

Analysis. 0·1072 g. gave 0·3053 g. CO_2; 0·0575 g. H_2O.

	C	H
Found	77·7 %	5·9 %
Calc. for $C_{13}H_{12}O_2$	78·0 %	6·0 %

Oxidation of 4-(4′-*methoxyphenoxy*) *toluene.* The compound was boiled for some hours under a reflux condenser with *N* potassium permanganate. The solution was treated with sulphur dioxide, cooled and extracted with ether; the ether was shaken out with sodium carbonate; the latter gave, on acidification, a small yield of 4-(4′-methoxyphenoxy) benzoic acid, which, on recrystallisation from dilute alcohol, formed colourless plates, M.P. 176·5°; when mixed with some of the acid degradation product $C_{14}H_{12}O_4$ the mixture melted at 176·5°.

(4-*Methoxyphenoxy*) *benzene*. This compound was prepared in a precisely similar manner to the 4-(4′-methoxyphenoxy) toluene by heating a mixture of 18·7 g. *p*-bromoanisol, 11·75 g. phenol, 5·6 g. potassium hydroxide and 0·1 g. copper bronze to 235° for 4–5 hours. In this case the reaction product could be distilled under ordinary pressure, giving a colourless mobile oil, B.P. 293–296°. The yield was 70 % of the theoretical.

Analysis. 0·1262 g. gave 0·3600 g. CO_2; 0·0730 g. H_2O.

	C	H
Found	77·8 %	6·4 %
Calc. for $C_{13}H_{12}O_2$	78·0 %	6·0 %

The compound was further characterised by the fact that on boiling with hydriodic acid it gave (4-hydroxyphenoxy) benzene, felted needles from water, M.P. 84°, a compound which had been previously prepared by Biltz [1896] in a different manner.

4-(4′-*Methoxyphenoxy*) *benzaldehyde*. 21 g. (4-methoxyphenoxy) benzene were mixed with 35 cc. benzene, cooled in ice and treated with 27·2 g. fresh and finely powdered aluminium chloride and 18 cc. of anhydrous hydrocyanic acid. The solution was contained in a flask attached to an efficient reflux condenser, the upper end of which was closed by a calcium chloride tube, and also to an inlet tube with a wide mouth through which a stream of dry hydrogen chloride could be led in; the hydrogen chloride was led in for 30 minutes at 0°, 30 minutes at the ordinary temperature, and finally for 3 hours at 40–45°; the reaction mixture was decomposed with ice and hydrochloric acid and then steam-distilled for about half an hour. After cooling, the oil was separated with the aid of ether and the ethereal solution dried; the aldehyde could be distilled under reduced pressure, but there was not much to be gained thereby owing to the small difference in boiling point between the aldehyde and the unchanged starting material. It was found better to concentrate the ethereal solution and to isolate the aldehyde by means of its insoluble bisulphite compound in the usual way. After decomposing the bisulphite compound, the aldehyde was extracted with ether and the solution dried and evaporated; the residual oil crystallised rapidly; it was drained from a small amount of adherent oil on a porous tile and was then sufficiently pure for further experiments. The yield was poor and variable, the average being 25–30 % and the highest recorded just under 50 % of the theoretical. On recrystallisation from light petroleum it formed colourless macroscopic needles or long narrow prisms. The melting point was 60·5°; when mixed with a sample of the natural aldehyde $C_{14}H_{12}O_3$ which had M.P. 59·5° the mixture melted at 60°. Under the same conditions as the natural aldehyde, the synthetic product yielded a *semicarbazone*, M.P. 211°, and an *oxime*, M.P. 75°. It was therefore completely identical with the natural degradation product.

Oxidation of 4-(4′-*methoxyphenoxy*) *benzaldehyde.* 0·45 g. of the aldehyde was dissolved in 4 cc. of acetone and treated with 0·23 g. potassium permanganate which was finely powdered and added in small portions. Slight warming occurred. When the permanganate was all used up the precipitate was filtered, washed with acetone and repeatedly extracted with water; on acidifying the aqueous extract about 0·4 g. of an acid was obtained, which on recrystallisation from aqueous alcohol formed colourless plates, M.P. 176·5°; the melting point of a mixture of this acid with the degradation product $C_{14}H_{12}O_4$ was 176°.

4-(4′-*Methoxyphenoxy*) *cinnamic acid.* 0·5 g. 4-(4′-methoxyphenoxy) benzaldehyde, 0·15 g. fused sodium acetate and 0·5 cc. acetic anhydride were heated for 18 hours at 150°; the reaction product was warmed with sodium carbonate solution and filtered from some oil. The sodium salt which crystallised out on cooling was filtered off, dissolved in hot water and the acid precipitated with hydrochloric acid. On recrystallisation from dilute alcohol it formed colourless glistening leaflets, M.P. 175·5°; when mixed with the acid $C_{16}H_{14}O_4$ the melting point was again 175·5°. The sodium and potassium salts of the synthetic acid, like those of the acid $C_{16}H_{14}O_4$, are very slightly soluble in cold water. The two acids appear, therefore, to be completely identical.

(A) *Synthesis of β-*[4-(4′-*hydroxyphenoxy*) *phenyl*]-*α-aminopropionic acid.* 2·4 g. 4-(4′-methoxyphenoxy) benzaldehyde, 0·5 g. glycine anhydride and 1·3 g. freshly fused sodium acetate were ground up together, treated with 2 cc. of freshly distilled acetic anhydride and heated in a tube attached to a reflux condenser for 6 hours at 120–130°. After cooling, the solid mass was shaken out and ground up first with water and then with alcohol. Finally, it was dried, ground up, and boiled out several times with ligroin, when it was pure enough for further experiment. The compound was extremely insoluble in all solvents; it could be crystallised from a large volume of glacial acetic acid, from which it separated in small yellow rectangular plates, M.P. 286–287°. The yield was 60 % of the theoretical.

23·0 mg. gave 1·16 mg. N (Micro-Kjeldahl).

Found	N 5·04 %
Calc. for $C_{32}H_{26}O_6N_2$	N 5·24 %

1 g. of the above compound was boiled with 10 cc. of hydriodic acid (sp.g. 1·7) and 1 g. red phosphorus for 4 hours, and then, with the addition of 1·2 g. red phosphorus, for a further 5 hours; the solution was filtered hot through asbestos, diluted with water, filtered from some tar and shaken out with ether. It was then concentrated almost to dryness *in vacuo*; the crystalline residue of the hydriodide of the amino-acid was dissolved in warm water and the hot solution treated with sodium acetate. The amino-acid crystallised out and was recrystallised by dissolving in warm dilute sulphuric acid and precipitating with sodium acetate. The yield was about 50 % of the theoretical. The product showed the same crystalline form, the same solubilities and the same reactions as desiodo-thyroxine. On heating it melted with decomposition at 253–254°.

Analysis. 22·8 mg. gave 1·1 mg. N (Micro-Kjeldahl).
18·5 mg. gave 1·72 cc. moist N at 17° and 754 mm. (Van Slyke).

	Total N	Amino-N
Found	4·8 %	5·3 %
Calculated	5·1 %	5·1 %

The *hydrochloride*, prepared in the same way as that of the natural product, formed colourless plates, M.P. 238–240°; when mixed with the hydrochloride of natural desiodo-thyroxine it melted at 237–239°.

0·1139 g. gave 0·0520 g. AgCl.

Found	Cl	11·3 %
Calculated	Cl	11·5 %

(B) 4-(4′-*Methoxyphenoxy*) *benzalhydantoin*. An intimate mixture of 4 g. 4-(4′-methoxyphenoxy) benzaldehyde, 1·72 g. hydantoin, and 4 g. fused sodium acetate was treated with 8 cc. glacial acetic acid and 0·4 cc. acetic anhydride, and boiled on the oil-bath under a reflux condenser for 3 hours. While still warm the mixture was poured into a mortar, and, when cold, was ground up with water, filtered and well washed. The yellow product was then dried, powdered and boiled out several times with ligroin. After this treatment it was pure enough for further experiment. The yield was 60 %. Recrystallised from glacial acetic acid, it formed pale yellow needles, M.P. 211°.

15·0 mg. gave 1·31 mg. N (Micro-Kjeldahl).

Found	N 8·7 %
Calc. for $C_{17}H_{16}O_5N_2$	N 9·0 %

4-(4′-*Methoxyphenoxy*) *benzylhydantoin*. The sodium compound of the above condensation product was very slightly soluble in water but was soluble in dilute alcohol. The reduction was therefore carried out as follows: 4·65 g. 4-(4′-methoxyphenoxy) benzalhydantoin were dissolved in 75 cc. 2 *N* sodium hydroxide and 200 cc. 95 % alcohol and treated with 150 g. of 2·5 % sodium amalgam which was broken into small pieces and added in five portions in the course of half an hour, the solution being well shaken; after standing for an hour, 100 cc. of water were added to the solution, which had become almost colourless, and the alcohol was distilled off under reduced pressure; the aqueous solution was cooled, filtered and cautiously neutralised with hydrochloric acid; after standing in ice for a few hours, the white crystalline precipitate was filtered off. Yield 4·0 g. It was recrystallised from dilute alcohol and formed colourless rectangular plates, M.P. 177·5°.

15·4 mg. gave 1·33 mg. N (Micro-Kjeldahl).

Found	N 8·6 %
Calc. for $C_{17}H_{18}O_5N_2$	N 9·0 %

β-[4-(4′-*Methoxyphenoxy*) *phenyl*]-*a*-*aminopropionic acid*. 2·5 g. 4-(4′-methoxyphenoxy) benzylhydantoin were refluxed for 6½ hours with 30 g. crystalline barium hydroxide and 50 cc. water; the hydantoin was very insoluble in the barium hydroxide solution and was recovered for the most part unchanged; the filtrate was made acid to Congo red with sulphuric acid, filtered from barium sulphate and the filtrate and washings treated with sodium acetate until no free mineral acid was present; on concentrating on the water-bath a minute amount of an amino-acid crystallised out. On recrystallisation it formed bunches of small needles, which, on heating, darkened at 210° and melted with decomposition at 220–221°.

18·6 mg. gave 0·9 mg. N (Micro-Kjeldahl).
16·7 mg. gave 1·34 cc. moist N at 17° and 754 mm. (Van Slyke).

	Total N	NH_2-N
Found	4·8 %	4·6 %
Calc. for $C_{16}H_{17}O_4N$	4·9 %	4·9 %

On boiling with hydriodic acid it gave a substance having the appearance of desiodo-thyroxine, but the amount was too small for identification. A better method of preparing the desired amino-acid consisted in simultaneous reduction, hydrolysis and demethylation of the 4-(4′-methoxyphenoxy) benzalhydantoin by boiling with hydriodic acid and red phosphorus.

4-(4′-*Hydroxyphenoxy*) *benzylhydantoin.* This compound was formed on boiling 4-(4′-methoxyphenoxy) benzalhydantoin for 1 hour with 10 parts of hydriodic acid (sp.g. 1·7) and 1 part of red phosphorus. Crystallised from very dilute alcohol it formed small colourless cubes, M.P. 245–246°.

15·5 mg. gave 1·38 mg. N (Micro-Kjeldahl).

Found	N 8·9 %
Calc. for $C_{16}H_{16}O_5N_2$	N 9·1 %

β-[4-(4′-*Hydroxyphenoxy*) *phenyl*]-*α*-*aminopropionic acid.* 2 g. 4-(4′-methoxyphenoxy) benzalhydantoin were boiled for 4 hours with 20 cc. hydriodic acid (sp.g. 1·7) and 2 g. red phosphorus; a further 3 g. red phosphorus was then added and the boiling continued for 5 hours more. The resulting solution was worked up in precisely the same manner as that described under the synthesis by the glycine anhydride method. There was obtained just under 1 g. of an amino-acid having the same solubilities, reactions and crystalline appearance as desiodo-thyroxine. It melted with decomposition at 252–253°.

Analysis. 0·1073 g. gave 0·2587 g. CO_2; 0·0543 g. H_2O.
20·8 mg. gave 1·02 mg. N (Micro-Kjeldahl).
20·0 mg. gave 1·80 cc. moist N at 17° and 754 mm. (Van Slyke).

	C	H	N (total)	N (amino)
Found	65·75	5·6	4·9	5·2
Calc. for $C_{15}H_{15}O_4N$	65·9	5·5	5·1	5·1

The *hydrochloride*, prepared as described above, had M.P. 238–240°, mixed with the hydrochloride of natural desiodo-thyroxine, 237–239°.

0·1263 g. gave 0·0576 g. AgCl.

Found	Cl 11·3 %
Calc. for $C_{15}H_{16}O_4NCl$	Cl 11·5 %

The identity of natural desiodo-thyroxine and the synthetic amino-acid is therefore established.

In conclusion I desire to express my sincere gratitude to Prof. G. Barger, F.R.S., for his continued interest in this work and for much valuable advice.

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