150. *The Synthesis of Thyroxine and Related Substances. Part IX.* Analogues of Thyroxine with Modified Side Chains.*

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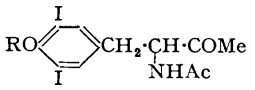
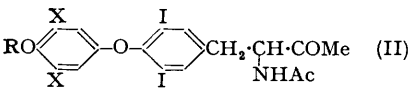
3 : 5-Di-iodothyronine and thyroxine have been converted into analogues of thyroxine with the alanine side chain replaced by other groups, in the hope of producing antagonists of the hormone.

3 : 5-Di-iodotyrosine, 3 : 5-di-iodothyronine, and thyroxine were converted by acetic anhydride in pyridine into the expected methyl α-acetamido-ketones, which were cyclised with phosphoryl chloride to the 2 : 5-dimethyl-oxazoles.

Exhaustive methylation and Hofmann degradation of 3 : 5-di-iodothyronine and thyroxine led to the corresponding methylated unsaturated acids (V; R = Me, X = H) and (V; R = Me, X = I) respectively. Demethylation and iodination of the former product yielded the acrylic acid analogue (V; R = H, X = I) of thyroxine. These cinnamic acids reacted with hydroxylamine to give either the oxime of the corresponding acetophenone or the β-amino-acid, or a mixture of both. β-Thyroxine † was made by iodination of 3 : 4-di-iodo-β-thyronine prepared in this way.

A modification of the molecule of essential amino-acids that has often led to the production of metabolic antagonists is the substitution of a methyl group (radius 2·0 Å) for the hydroxyl (radius 1·7 Å) of the carboxyl group. Ketones of this kind have been shown to inhibit the effects of *p*-aminobenzoic acid (Auhagen, *Z. physiol. Chem.*, 1942, **274**, 48; Kuhn, Moller, Wendt, and Beinert, *Ber.*, 1942, **75**, 711), nicotinic acid (Woolley, *J. Biol. Chem.*, 1945, **157**, 455; Gaebler and Beher, *ibid.*, 1951, **188**, 343), and pantothenic acid (Woolley and Collyer, *J. Biol Chem.*, 1945, **159**, 263) to varying degrees in bacteria or mammals; and the methyl α-amino-ketones corresponding to leucine, valine, *iso*leucine, and tyrosine prevent regrowth of the tails of tadpoles after amputation, although admittedly there is no proof that this is due to their direct interference with the metabolism of these amino-acids (Lehmann, Bretscher, Kühne, Sorkin, Erne, and Erlenmeyer, *Helv. Chim. Acta*, 1950, **33**, 1217). The hydrolysis of *N*-benzoyl- and *N*-acetyl-L-tyrosinamide catalysed by chymotrypsin is specifically inhibited by 3-acetamido-4-phenyl- and 3-acetamido-4-*p*-hydroxyphenylbutan-2-one (Kaufman and Neurath, *J. Biol. Chem.*, 1949, **181**, 623).

(I) 

 (II)

Although the few tested modifications of the thyroxine side chain have produced compounds with varying degrees of thyroxine activity (Frieden and Winzler, *J. Biol. Chem.*, 1948, **176**, 155), we attempted the preparation of the methyl ketone corresponding to thyroxine as a possible antagonist.

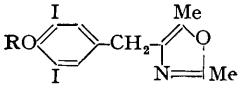
The amino-acids, 3 : 5-di-iodo-L-tyrosine, 3 : 5-di-iodo-L-thyronine, and L-thyroxine, reacted in boiling pyridine with a large excess of acetic anhydride (Levene and Steiger, *J. Biol. Chem.*, 1927, **74**, 689; 1928, **79**, 95; Dakin and West, *ibid.*, 1928, **78**, 91, 745; Cleland and Niemann, *J. Amer. Chem. Soc.*, 1948, **71**, 841), evolving carbon dioxide and producing the DL-acetamido-acetoxy-ketones (I; R = Ac), (II; R = Ac; X = H), and (II; R = Ac; X = I) respectively. The compound (I; R = Ac) could not be induced to crystallise, but treatment with hot sodium carbonate solution yielded the crystalline hydroxy-compound (I; R = H).

* Part VIII, *J.*, 1951, 2467.

† The β-amino-acids, β-amino-β-(4-*p*-hydroxyphenoxyphenyl)propionic acid and β-amino-β-[4-(4′-hydroxy-3′ : 5′-di-iodophenoxy)-3 : 5-di-iodophenyl]propionic acid (VII; R = H, X = I), isomeric with the α-amino-acids, thyronine and thyroxine, are referred to here as "β-thyronine" and "β-thyroxine" respectively.

Cyclisation of the methyl α-acetamido-ketone grouping in these compounds gave access to a series of analogues containing the 2 : 5-dimethyloxazole group. When the ketone (II; R = Ac; X = H) from di-iodothyronine was treated with sulphuric acid, the usual dehydrating agent for such cyclisations (Wiley, *Chem. Reviews*, 1945, **37**, 40), the resulting oxazole contained a sulphonic acid group, presumably next to the hydroxyl group, *i.e.*, to give (IV; X = SO_3H; Y = H).

Cyclisation of the three acetamido-ketones proceeded smoothly, however, in phosphoryl chloride on the steam-bath. Hydrolysis of a solution of (I; R = H) in phosphoryl chloride gave a clear aqueous solution from which the organic matter could be extracted with chloroform only after boiling, suggesting that the oxazole (III; R = H) had originally been present as a phosphoric ester. In the same way, heating the reaction mixture of (II; R = Ac; X = H) and phosphoryl chloride with water (to hydrolyse the *O*-acetyl group) gave (IV; X = Y = H). Similar treatment of the tetra-iodo-compound (II; R = Ac; X = I) yielded a substance thought to be solvated (IV; X = Y = I) until the authentic hydroxy-compound was made by iodination of (IV; X = Y = H) and found



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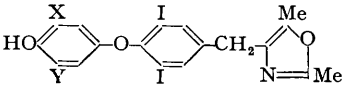

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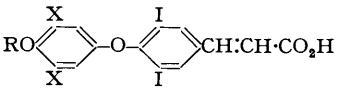


(V)

different. The product of direct cyclisation was then shown to be the acetyl derivative of (IV; X = Y = I): it contained an acetyl group and gave a negative Kendall test with nitrous acid and ammonia. The survival of the acetyl group in the tetra-iodo-, but not in the di-iodo-, compound is probably due to the lower solubility of the former in aqueous acid.

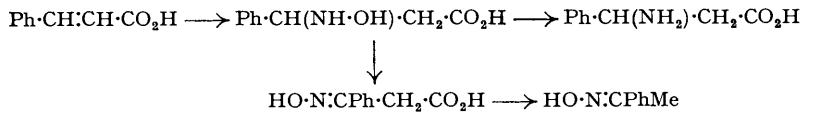
As *N*-acetyl-*O*-benzyl-3 : 5-di-iodotyrosine antagonises thyroxine in tadpoles (Woolley, *J. Biol. Chem.*, 1946, **164**, 11; Frieden and Winzler, *ibid.*, 1949, **179**, 423), the acetamido-ketone (I; R = H) from di-iodotyrosine was condensed with benzyl chloride in ethyl methyl ketone containing potassium carbonate, to give the benzyl ether (I; R = $C_6H_5{\cdot}CH_2$) The corresponding oxazole (III; R = H), however, was recovered unchanged after the same treatment, although it condensed normally when the sodium salt was boiled with benzyl chloride in ethanol.

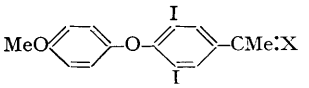
Acid hydrolysis of (II; R = Ac or H; X = I) under various conditions gave gums from which no crystalline salt of the amino-ketone corresponding to thyroxine could be isolated.

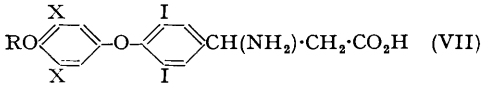
The acrylic acid analogue of thyroxine (V; R = H, X = I), the methyl ether of which was prepared by Hofmann degradation of thyroxine during the classical work on the hormone (Harington and Barger, *Biochem. J.*, 1927, **21**, 169), as well as being a versatile starting material for further transformations, is itself of some interest as a possible antagonist, by analogy with the competition of indolylacrylic acid with tryptophan in bacteria (Fildes, *ibid.*, 1938, **32**, 1600). Exhaustive methylation and alkaline treatment of thyroxine, following Corti's method for the Hofmann degradation of tyrosine (*Helv. Chim. Acta*, 1949, **32**, 681), gave (V; R = Me, X = I) in high yield. This substance could not be demethylated by hydriodic acid in acetic acid owing to its extremely low solubility. With phenol as solvent, demethylation occurred, but the product appeared to be chiefly (V; R = X = H), showing that reduction or trans-iodination with the solvent had also taken place. The di-iodo-compound (V; R = Me, X = H), got by Hofmann degradation of 3 : 5-di-iodothyronine, also in excellent yield, was demethylated, however, without difficulty. The product was then iodinated to the acrylic acid analogue of thyroxine (V; R = H, X = I). After this work was complete the synthesis of (V; R = H, X = H and I) from 3 : 5-di-iodo-4-*p*-methoxyphenoxybenzaldehyde was reported by Wawzonek, Wang, and Lyons (*J. Org. Chem.*, 1950, **15**, 593); the melting or decomposition points of both compounds were given as about 40° below those reported here.

A little known reaction of cinnamic acid is its conversion by hydroxylamine into a

mixture of β-amino-β-phenylpropionic acid and acetophenone oxime (Posner, *Ber.*, 1905, **38**, 2320; *Annalen*, 1912, **389**, 120; Steiger, *Org. Synth.*, 1942, **22**, 26):

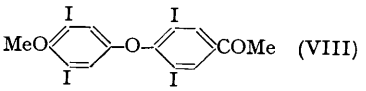


(VI) 

 (VII)

When boiled with a solution of hydroxylamine in alcohol–dioxan the di-iodocinnamic acid (V; R = Me, X = H) gave a mixture of the ketoxime (VI; X = N·OH) (36% yield) and 3 : 5-di-iodo-β-thyronine methyl ether (VII; R = Me, X = H) (25% yield). The constitution of both products was definitely established. The oxime reacted with 2 : 4-dinitrophenylhydrazine hydrochloride with formation of the 2 : 4-dinitrophenylhydrazone, and on hydrolysis gave the ketone (VI; X = O), degraded by iodine in alkaline solution to iodoform and 3 : 5-di-iodo-4-*p*-methoxyphenoxybenzoic acid, identical with an authentic sample (Part I, *J.*, 1949, S 185). The second product (VII; R = Me, X = H) behaved as a typical β-amino-acid. It yielded an acetyl derivative, and lost ammonia above its melting point, regenerating the methoxy-cinnamic acid (V; R = Me, X = H). Treatment with hydriodic acid in acetic acid resulted in loss of ammonia as well as demethylation, to give the hydroxy-cinnamic acid (V; R = X = H).

Reaction of the tetra-iodocinnamic acid (V; R = Me, X = I) with hydroxylamine under similar conditions led, on the other hand, to the oxime of the methyl ketone (VIII) as the only isolated product (41%); even a small proportion of β-amino-acid could hardly have been overlooked because of the very low solubility of such a compound. The oxime could not be obtained crystalline, nor initially could the ketone (VIII) itself. The product was therefore converted into the 2 : 4-dinitrophenylhydrazone, which was purified by chromatography and then hydrolysed back to the free ketone with acetone containing hydrochloric acid. A second experiment, in which the product was hydrolysed directly to the ketone, gave a lower yield.

 (VIII)

One of the objectives of this work was the production of β-thyroxine (VII; R = H, X = I) and its derivatives. After the failure of the last reaction to lead to any of the methyl ether, the shortest route to β-thyroxine seemed to be to iodinate 3 : 5-di-iodo-β-thyronine. The latter could not be got from its methyl ether, but resulted, though in rather poor yield, from the reaction of the hydroxycinnamic acid (V; R = X = H) with hydroxylamine. It was smoothly iodinated with potassium tri-iodide in aqueous ethylamine (Part VI; *J.*, 1950, 840) to β-thyroxine.

The antithyroid potencies of the above compounds were examined by Maclagan and Sheehan's method (*J. Endocrinol.*, 1950, **6**, 456), but none showed significant activity.

Experimental

DL-3-*Acetamido*-4-(4-*hydroxy*-3 : 5-*di-iodophenyl*)*butan*-2-*one* (I; R = H).—3 : 5-Di-iodo-L-tyrosine (25 g.) was dissolved in a mixture of dry pyridine (150 ml.) and acetic anhydride (60 ml.), and the solution was heated under reflux for 2½ hours. No further carbon dioxide evolution could be detected after 2 hours. Chloroform (100 ml.) was added to the cooled pyridine solution, and the whole stirred below 20° with excess of 2N-hydrochloric acid to remove the pyridine. A similar treatment of the chloroform solution with 2N-sodium carbonate hydrolysed excess of acetic anhydride, the stirring being continued for 1 hour. The chloroform was dried azeotropically by concentration to about 50 ml. and passed down an alumina column. Most of the tarry impurities were adsorbed and the product passed straight through as a yellow solution.

This was taken to dryness *in vacuo*, the gum was dissolved in a little ethanol, and anhydrous sodium carbonate (40 g.) was added as a strong aqueous solution. After steam had been blown in for 1 hour, the cooled aqueous solution was decanted from the small tarry residue, acidified, and extracted with chloroform (3 × 50 ml.) which was concentrated to dryness. The resulting gum crystallised from ethyl acetate, to give nearly white needles of the *ketone*, m. p. 150—152° (12·0 g., 44%) (Found: C, 30·4; H, 2·8; I, 54·1. $C_{12}H_{13}O_3NI_2$ requires C, 30·5; H, 2·7; I, 53·7%).

DL-3-*Acetamido*-4-(4-p-*acetoxyphenoxy*-3 : 5-*di-iodophenyl*)*butan*-2-*one* (II; R = Ac, X = H).—3 : 5-Di-iodo-L-thyronine was treated as above. After removal of the pyridine and excess of acetic anhydride the product was chromatographed in benzene on alumina. Elution with a mixture of benzene and acetone (equal parts by volume) removed a yellow band. On evaporation of the eluate a gum remained which was crystallised twice from ethyl acetate and *cyclo*hexane, yielding the *ketone* as white crystals, m. p. 126—130° (4·8 g. from 10 g., 42%) (Found: C, 39·7; H, 3·5; I, 41·0. $C_{20}H_{19}O_5NI_2$ requires C, 39·6; H, 3·2; I, 41·8%).

The ketone (0·25 g.) was dissolved in ethanol (3 ml.) with gentle heat and added to a solution of 2 : 4-dinitrophenylhydrazine (0·09 g.) in sulphuric acid (1 ml.) and ethanol (3 ml.). The mixture was warmed on the steam-bath for 30 minutes. Addition of water precipitated the 2 : 4-*dinitrophenylhydrazone* which was crystallised twice from ethanol to yield bright yellow crystals, m. p. 130° (Found: N, 8·7; I, 32·4. $C_{24}H_{21}O_6N_5I_2$ requires N, 8·8; I, 32·1%).

DL-3-*Acetamido*-4-[4′-(4″-*acetoxy*-3″ : 5″-*di*-*iodophenoxy*)-3′ : 5′-*di*-*iodophenyl*]*butan*-2-*one* (II; R = Ac, X = I).—L-Thyroxine was treated as before and the pyridine and acetic anhydride were removed. The chloroform solution was evaporated to dryness and the gum crystallised from ethyl acetate, to yield white crystals of the *ketone*, m. p. 223—226° (10·5 g. from 25 g.; 38%) (Found: C, 28·1; H, 2·1; I, 58·8. $C_{20}H_{17}O_5NI_4$ requires C, 28·0; H, 2·0; I, 59·1%).

DL-3-*Acetamido*-4-[4′-(4″-*hydroxy*-3″ : 5″-*di*-*iodophenoxy*)-3′ : 5′-*di*-*iodophenyl*]*butan*-2-*one* (II; R = H, X = I).—The mother-liquor from the previous preparation was concentrated to dryness *in vacuo* and chromatographed in benzene on alumina. The yellow solution which passed through contained nothing of consequence, but with a mixture of benzene and acetone (equal parts by volume) a brown fraction was eluted. On removal of the solvent the residue crystallised. Recrystallisation from aqueous dioxan yielded the *ketone* as white needles, m. p. 253—254° with frothing (3·5 g., total yield including acetoxy-compound, 51%) (Found: C, 29·3; H, 2·3; I, 56·1. $C_{18}H_{15}O_4NI_4$ requires C, 29·2; H, 2·6; I, 56·1%).

4-(4-*Hydroxy*-3 : 5-*di*-*iodobenzyl*)-2 : 5-*dimethyloxazole* (III; R = H).—DL-3-Acetamido-4-(4-hydroxy-3 : 5-di-iodophenyl)butan-2-one (10 g.) was warmed in phosphorus oxychloride (15 ml.) on the steam-bath for 1 hour. Dissolution occurred rapidly and the mixture darkened somewhat. After cooling, the solution was poured carefully into water (150 ml.) to hydrolyse the phosphorus oxychloride. No special precautions were taken to prevent heating, and almost all the product remained in solution. The strongly acid liquid was then boiled under reflux for 1 hour, cooled, and extracted with chloroform (3 × 50 ml.). Some tarry matter remained undissolved. The chloroform was removed *in vacuo* and the residual gum crystallised from ethyl acetate. The *oxazole* formed white needles, m. p. 200—201° (decomp.) (6·8 g., 71%) (Found: C, 31·7; H, 2·3; I, 55·4. $C_{12}H_{11}O_2NI_2$ requires C, 31·7; H, 2·4; I, 55·8%).

4-(4-p-*Hydroxyphenoxy*-3 : 5-*di*-*iodobenzyl*)-2 : 5-*dimethyloxazole* (IV; X = Y = H).—DL-3-Acetamido-4-(4-*p*-acetoxyphenoxy-3 : 5-di-iodophenyl)butan-2-one (5 g.) was heated in phosphorus oxychloride (15 ml.) on the steam-bath for 3 hours. No darkening occurred. After hydrolysis of the phosphorus oxychloride in water (100 ml.), the mixture was heated on the steam-bath for 1 hour to complete the hydrolysis of the *O*-acetyl linkage. The white solid *oxazole* was filtered off and crystallised from aqueous ethanol, to give white crystals, m. p. 211—213° (3·6 g., 80%) (Found: C, 39·4; H, 2·6; I, 46·4. $C_{18}H_{15}O_3NI_2$ requires C, 39·5; H, 2·8; I, 46·4%).

4-[4′-(4″-*Hydroxy*-3″-*sulphophenoxy*)-3′ : 5′-*di*-*iodobenzyl*]-2 : 5-*dimethyloxazole* (IV; X = SO_3H, Y = H).—The ketone (5 g.) was dissolved in sulphuric acid (5 ml.) at *ca.* 40°, and the solution was set aside for 3 hours. On its being poured into water (40 ml.), a brown oil was precipitated, which solidified slowly when heated on a steam-bath. The nearly white *acid* was crystallised twice from aqueous ethanol, and then melted at 222°, sintering from 177° (Found: I, 40·7; S, 5·4; $C_{18}H_{15}O_6NSI_2$ requires I, 40·5; S, 5·1%).

4-[4′-(4″-*Acetoxy*-3″ : 5″-*di*-*iodophenoxy*)-3′ : 5′-*di*-*iodobenzyl*]-2 : 5-*dimethyloxazole*.—DL-3-Acetamido-4-[4′-(4″-acetoxy-3″ : 5″-di-iodophenoxy)-3′ : 5′-di-iodophenyl]butan-2-one (5 g.), dissolved in phosphorus oxychloride (15 ml.), was heated on the steam-bath for 3 hours. The phosphorus oxychloride was hydrolysed in water (100 ml.), and the mixture heated on the steam-

bath for 1 hour. When the solid product was dissolved in ethanol (200 ml.) and concentrated to 20 ml., a little tar was precipitated. The clear liquid was decanted and set aside till crystallisation occurred. The *oxazole* formed nearly white crystals, m. p. 209—211°, sintering at 195° (2·2 g., 48%) (Found: C, 28·6; H, 2·4; I, 59·6. $C_{20}H_{15}O_4NI_4$ requires C, 28·6; H, 1·8; I, 60·3%).

4-[4′-(4″-*Hydroxy*-3″ : 5″-*di-iodophenoxy*)-3′ : 5′-*di-iodobenzyl*]-2 : 5-*dimethyloxazole* (IV; X = Y = I).—Iodine [1·94 ml. of solution in excess of aqueous potassium iodide; 245 g. of iodine per l. (4 atoms)] was added dropwise with stirring to a solution of the di-iodo-compound in 20% ethylamine solution (5 ml.). A pale yellow solid crystallised. The mixture was diluted with water and acidified. The very pale yellow solid that was filtered off crystallised poorly from most solvents. In acetic acid it became oily and dissolved: crystals then separated on scratching. Recrystallisation from acetic acid gave almost colourless crystals of the *tetraiodo*-compound, m. p. 182—183° (decomp.), depressed by the preceding compound (Found: C, 27·6; H, 1·9; I, 61·4. $C_{18}H_{13}O_3NI_4,\frac{1}{2}C_2H_4O_2$ requires C, 27·5; H, 1·8; I, 61·2%). Recrystallisation then from alcohol gave crystals, m. p. 197—199° (decomp.) (Found: C, 28·7; H, 2·2; N, 1·7. $C_{18}H_{13}O_3NI_4,C_2H_6O$ requires C, 28·4; H, 2·3; N, 1·7%).

4-(4-*Benzyloxy*-3 : 5-*di-iodobenzyl*)-2 : 5-*dimethyloxazole* (III; R = CH_2Ph).—The related hydroxy-compound (2 g.) was dissolved in dry ethanol (50 ml.) containing sodium (0·1 g., 1 atom). Benzyl chloride (0·5 ml., 1 mol.) was added and the solution was heated under reflux for 4 hours. The precipitated sodium chloride was removed by filtration, and the solution taken to dryness *in vacuo*. The gum was extracted with warm *cyclo*hexane from which the *ether* crystallised as fine white needles of m. p. 98—99° (1·32 g., 55%) (Found: C, 41·6; H, 3·0; I, 47·2. $C_{19}H_{17}O_2NI_2$ requires C, 41·8; H, 3·1; I, 46·6%).

DL-3-*Acetamido*-4-(4-*benzyloxy*-3 : 5-*di-iodophenyl*)*butan*-2-*one* (I; R = CH_2Ph).—The hydroxy-compound (2 g.) was dissolved in ethyl methyl ketone (50 ml.); benzyl chloride (0·6 ml., 1·1 mols.) and potassium carbonate (1 g., *ca.* 3½ equivs.) were added and the solution was refluxed for 3 hours. The inorganic material was removed by filtration and the solvent removed *in vacuo*. Crystallisation of the gum from ethanol yielded the *ether* as white needles, m. p. 177—178° (1·4 g., 58%) (Found: C, 40·7; H, 3·6; I, 45·6. $C_{19}H_{19}O_3NI_2$ requires C, 40·5; H, 3·4; I, 45·1%). The substance was insoluble in alkali and gave a negative Kendall test.

3 : 5-*Di-iodo*-4-p-*methoxyphenoxycinnamic Acid* (V; R = Me, X = H).—3 : 5-Di-iodo-L-thyronine (50 g.) was dissolved in water (180 ml.) containing sodium hydroxide (8 g.) and stirred while methyl sulphate was added slowly until the pH was about 5 (*ca.* 9 ml.). Sodium hydroxide solution (40%; 15 ml.) was added and methyl sulphate (*ca.* 18 ml.) until the pH was again about 5. Much solid was precipitated. More sodium hydroxide (40%; 18 ml.) was added, followed by methyl sulphate (36 ml.), and the mixture was heated on the steam-bath for 1 hour. A brown oil was precipitated, the pH of the supernatant fluid being 5. A large excess of sodium hydroxide (40%; 80 ml.) was finally introduced and the mixture heated on the steam-bath for 3 hours. The mixture at the end of this period was a thick sludge of fine white needles in a brownish liquid and there was very little smell of trimethylamine.

The needles of sodium salt were filtered off and stirred with 5N-hydrochloric acid to generate the free acid as very fine needles, m. p. 250—252° (46·6 g., 94%). After recrystallisation from a large bulk of ethanol the prisms melted at 253—255° (Found: C, 36·6; H, 2·6; I, 48·3. Calc. for $C_{16}H_{12}O_4I_2$: C, 36·8; H, 2·3; I, 48·6%).

3 : 5-*Di-iodo*-4-(3 : 5-*di-iodo*-4-*methoxyphenoxy*)*cinnamic Acid* (V; R = Me, X = I).—Starting with L-thyroxine a similar process to the above yielded the required compound. In order to dissolve the starting material, equal quantities of ethanol and water were used as solvent. White crystals, m. p. 292—300° (some decomp.), were obtained from aqueous dioxan (47 g. from 50 g.; 94%) (Found: I, 64·9. Calc. for $C_{16}H_{10}O_4I_4$: I, 65·6%).

4-p-*Hydroxyphenoxy*-3 : 5-*di-iodocinnamic Acid* (V; R = X = H).—The foregoing methoxy-acid (10 g.) was dissolved in a mixture of acetic acid (200 ml.) and hydriodic acid (57%; 30 ml.) and the whole refluxed for 2 hours. Some crystalline solid which came out of solution during the heating was filtered off next morning. The crystals were nearly colourless after being washed with acetic acid, and melted at 290—300° (decomp.) (7·2 g., 73%). Crystallisation from aqueous dioxan formed white needles, m. p. 295—296° with some decomposition (Found: C, 35·3; H, 2·2; I, 49·2. Calc. for $C_{15}H_{10}O_4I_2$: C, 35·4; H, 2·0; I, 50·0%).

4-(4-*Hydroxy*-3 : 5-*di-iodophenoxy*)-3 : 5-*di-iodocinnamic Acid* (V; R = H, X = I).—The di-iodo-acid (2 g.) was dissolved in ethylamine solution (16% in water; 60 ml.) which was stirred during the addition, in 5 minutes, of iodine solution (1·9N in excess of sodium iodide; 2·3 ml., 4·4 atoms). Stirring was continued for 1 hour during which the product crystallised.

This was filtered off and stirred with 5N-hydrochloric acid to decompose the ethylamine salt. Crystallisation from a large bulk of acetic acid yielded nearly colourless needles, which finally decomposed at 270°, beginning to lose iodine at 255° (2·0 g., 66%) (Found: C, 24·0; H, 1·35; I, 67·5. Calc. for $C_{15}H_8O_4I_4$: C, 23·7; H, 1·1; I, 66·8%).

Reaction of 3 : 5-*Di-iodo*-4-p-*methoxyphenoxycinnamic Acid with Hydroxylamine.*—Hydroxylamine hydrochloride (3·66 g., 2·2 mols.) in water (7 c.c.) was added to a solution of sodium (1·2 g.; 2·2 atoms) in alcohol (100 c.c.), and the flask was washed with alcohol (5 c.c.) which was also added. The iodocinnamic acid (12·5 g., 1 mol.) and dioxan (30 c.c.) were added to the solution of hydroxylamine, from which the precipitated sodium chloride had been filtered off. A clear solution was obtained on boiling. After about 5 hours' boiling fine crystals of β-*amino*-β-(3 : 5-*di-iodo*-4-p-*methoxyphenoxyphenyl*)*propionic acid* began to separate. After 24 hours' boiling the mixture was cooled and the crystals of β-amino-acid (3·2 g., 25%) were filtered off and washed with water and alcohol. They melted with decomposition at 228—230°. Recrystallisation from pyridine raised the m. p. to 230—231°, with evolution of ammonia. The clear melt recrystallised at about 220° to fine needles of the corresponding cinnamic acid (V; R = Me, X = H), which did not completely remelt till 245° (Found: C, 35·7; H, 3·0; I, 47·2. $C_{16}H_{15}O_4NI_2$ requires C, 35·65; H, 2·8; I, 47·1%).

The β-amino-acid (0·6 g.) was dissolved in N-sodium hydroxide (10 c.c.), and acetic anhydride (0·6 c.c.) was added to the suspension of sodium salt that separated. Enough alkali was added at the same time to keep the solution alkaline. The clear solution was then acidified, and the precipitated β-*acetamido-acid* recrystallised from aqueous acetic acid. The fine colourless crystals sintered from 210° and melted at 248—251° (Found: C, 36·9; H, 2·9; I, 44·2. $C_{18}H_{17}O_5NI_2$ requires C, 37·2; H, 2·8; I, 43·7%)

After ¾ hour's boiling a solution of β-amino-acid (0·25 g.) in acetic acid (2 c.c.) and hydriodic acid (1 c.c.) was cooled, diluted with water, and neutralised with ammonia. The very pale yellow precipitate (0·22 g.) was filtered off and recrystallised from aqueous acetic acid as small rosettes of needles, m. p. (alone and mixed with 4-*p*-hydroxyphenoxy-3 : 5-di-iodocinnamic acid) 295° (decomp.), beginning to sinter at 260°.

3 : 5-*Di-iodo*-4-p-*methoxyphenoxyacetophenone Oxime.*—The filtrate after removal of the β-amino-acid from the reaction mixture was poured into water (2 l.) and twice extracted with chloroform, which was then washed with N-sodium carbonate and water. The residue remaining after evaporation of the chloroform was recrystallised from toluene–light petroleum (b. p. 100—120°), to give the *oxime* (3·55 g., 36%), m. p. 179—181° after sintering from 175°. After two more crystallisations from toluene–light petroleum (charcoal) and one from methyl cyanide, colourless blades (1·8 g.) were obtained, m. p. 183—184° after sintering from 180° (Found: C, 35·8; H, 2·8; I, 49·5. $C_{15}H_{13}O_3NI_2$ requires C, 35·4; H, 2·6; I, 49·8%).

The oxime (0·2 g.) and 2 : 4-dinitrophenylhydrazine (0·08 g., 1 mol.) in alcohol (10 c.c.) and concentrated hydrochloric acid (0·5 c.c.) were boiled. Yellow needles soon began to separate. After ½ hour the mixture was cooled and the 2 : 4-*dinitrophenylhydrazone* (0·25 g., 94%) was filtered off; it formed orange-yellow needles (from toluene), m. p. 225° (Found: N, 8·1; I, 37·0. $C_{21}H_{16}O_6N_4I_2$ requires: N, 8·3; I, 37·65%).

A solution of the oxime (0·5 g.) in alcohol (10 c.c.) and hydrochloric acid (1 c.c.) was boiled for 1 hour. After addition of water the solution deposited crystals of the *ketone* (0·43 g., 89%) on cooling. Recrystallisation from aqueous *iso*propanol and then from aqueous acetic acid gave colourless needles, m. p. 141—142° (Found: C, 36·6; H, 2·3; I, 51·4. $C_{15}H_{12}O_3I_2$ requires C, 36·4; H, 2·45; I, 51·4%).

To this ketone (50 mg.) in dioxan (0·6 c.c.) and 2N-sodium hydroxide (0·5 c.c.), iodine (0·32 c.c.; 245 g./l.; 6 atoms) in potassium iodide solution was added by drops, which were rapidly decolourised. The mixture was kept at about 60° for ¼ hour and then diluted with water. A small amount of solid with the characteristic smell of iodoform was filtered off. Acidification of the filtrate produced fine needles of 3 : 5-di-iodo-4-*p*-methoxyphenoxybenzoic acid (40 mg., 80%), which began to sinter at about 200° and finally melted at 234°. An authentic sample (Part I, *J.*, 1949, S 185) showed identical behaviour on being heated alone or mixed.

3 : 5-*Di-iodo*-4-(3 : 5-*di-iodo*-4-*methoxyphenoxy*)*acetophenone.*—(*a*) Hydroxylamine hydrochloride (1·58 g., 2·2 mols.) in water (3 c.c.) and alcohol (2 c.c.) was added to sodium (0·52 g., 2·2 atoms) in alcohol (50 c.c.). After filtration of the sodium chloride the solution was added to 3 : 5-di-iodo-4-(3 : 5-di-iodo-4-methoxyphenoxy)cinnamic acid (8·0 g., 1 mol.) in dioxan (70 c.c.). After 24 hours' boiling the solution was concentrated and filtered from a small amount of gelatinous stuff after dilution with benzene. Evaporation of the solvent left a gum that did not crystallise. It was therefore dissolved in alcohol (150 c.c.) containing hydrochloric acid (7 c.c.)

and 2 : 4-dinitrophenylhydrazine (1·9 g., 0·9 mol.), and boiled for 1 hour. The orange-yellow powder (4·7 g.) was removed from the cooled solution, washed, and dried. It was then dissolved in dioxan (60 c.c.) and benzene (120 c.c.) and chromatographed on activated alumina. The column was developed with dioxan–benzene (1 : 2), the clear yellow solution passing through and leaving greenish, orange, and brown bands on the column. The eluate was washed with water and the resulting benzene solution concentrated to small volume. Light petroleum (b. p. 100—120°) was added to the boiling residue till crystals began to appear. On cooling, the 2 : 4-*dinitrophenylhydrazone* was filtered off (3·9 g., 41%). It separated from chlorobenzene–light petroleum (b. p. 100—120°) in orange crystals m. p. 264—266° (Found : C, 27·4; H, 1·8; I, 54·4. $C_{21}H_{14}O_6N_4I_4$ requires C, 27·2; H, 1·5; I, 54·8%).

A solution of this hydrazone (150 mg.) in acetone (15 c.c.), dioxan (5 c.c.) and hydrochloric acid (0·75 c.c.) was boiled for 2 hours, cooled, and diluted with water till just cloudy. After a few minutes the pale yellow crystals of the *ketone* that had separated were filtered off; they had m. p. 223—226° after sintering from 205° (95 mg., 80%). Further dilution of the mother-liquor with water gave orange fibres of acetone 2 : 4-dinitrophenylhydrazone, m. p. 124—126° (30 mg., 80%). After crystallisation from light petroleum (b. p. 100—120°) the pale yellow needles of ketone sintered at 220° and melted at 224—226° (Found : C, 24·6; H, 1·5; I, 68·4. $C_{15}H_{10}O_3I_4$ requires C, 24·1; H, 1·3; I, 68·1%). After chromatography on alumina the ketone formed colourless blades of unchanged m. p.

(*b*) The cinnamic acid (7·1 g.) was boiled for 24 hours in a solution of hydroxylamine, made from the hydrochloride (1·40 g.) and sodium (0·46 g.), in alcohol (40 c.c.) and dioxan (60 c.c.) as before. After filtration from a little sludge the solution was poured into water, which was twice extracted with benzene. Evaporation of the solvent left a residue not easily recrystallised. So it was dissolved again in benzene and run down an alumina column. The first fractions separated from benzene–light petroleum (b. p. 100—120°) in rosettes of colourless blades (1·8 g., 26%), m. p. 220—224° after sintering from 216°, unchanged when mixed with ketone made from the 2 : 4-dinitrophenylhydrazone.

DL-3 : 5-*Di-iodo*-β-*thyronine*.—To 4-*p*-hydroxyphenoxy-3 : 5-di-iodocinnamic acid (5·0 g.) in dioxan (40 ml.) was added a solution of hydroxylamine, made from the hydrochloride (1·51 g., 2·2 mols.) in water (2 ml.) and alcohol (10 ml.) and sodium (0·50 g., 2·2 atoms) in alcohol (25 ml.). Having been boiled for 24 hours the mixture was filtered and the white solid (0·95 g.) was recrystallised from 50% alcohol. The resulting 3 : 5-*di-iodo*-β-*thyronine* sintered at 230° and melted with decomposition at 303° (Found : C, 34·5; H, 2·6; N, 2·8; I, 48·3. $C_{15}H_{13}O_4NI_2$ requires C, 34·3; H, 2·5; N, 2·7; I, 48·4%).

DL-β-*Thyroxine*.—3 : 5-Di-iodo-β-thyronine (0·32 g.) was dissolved by gentle warming in 33% ethylamine (4 ml.) and water (8 ml.). A solution of iodine (1·0 ml. of 2·76% : 4·4 atoms) in excess of aqueous potassium iodide was then added dropwise. After ½ hour the pH was brought to 6 with dilute acetic acid. The sticky brown precipitate solidified and was then decolourised with sodium hydrogen sulphite solution. It was dissolved in a large volume of methanol, which was filtered and concentrated. On cooling, β-*thyroxine* separated, having m. p. 211° (decomp.) (Found : N, 1·6; I, 65·7. $C_{15}H_{11}O_4NI_4$ requires N, 1·8; I, 65·4%).

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