715. *The Synthesis of Thyroxine and Related Substances. Part V. A Synthesis of* L-*Tyrosine from* L-*Tyrosine.**

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The synthesis of L-thyroxine from L-tyrosine by an extension of the methods described in Parts II and III of this series (this vol., pp. S 190, S 199) has been investigated. In preliminary experiments, the amino-acid grouping was protected by inclusion in a hydantoin ring and it was shown that, although racemisation occurred in varying degree at some of the stages, none of the essential reactions was completely incompatible with the retention of configuration. Protection of the amino-group by acetylation and the carboxy-group by esterification has made possible a synthesis of L-thyroxine, which was obtained in 26% overall yield from L-tyrosine without loss of optical activity.

The natural occurrence of thyroxine in an optically active form was first demonstrated by Harington and Salter (*Biochem. J.*, 1930, **24**, 456), who employed enzymic hydrolysis to liberate

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the compound from the protein in which it occurs in the thyroid gland and so avoided the racemisation that results from alkaline hydrolysis. A similar result was obtained by Foster, Palmer, and Leland (*J. Biol. Chem.*, 1936, **115**, 467), who employed acid hydrolysis after a preliminary enzymic digestion of the gland. The amino-acid was assigned to the normal L-series by Canzanelli, Harington, and Randall (*Biochem. J.*, 1934, **28**, 68), who prepared thyronine from both natural thyroxine and L-tyrosine and found that the samples had similar rotations. Harington (*ibid.*, 1928, **22**, 1429) also prepared L-thyroxine from synthetic 3 : 5-di-iodo-DL-thyronine, which was resolved through the L-1-phenylethylamine salt of its *N*-formyl derivative, the 3 : 5-di-iodo-L-thyronine then being iodinated.

More recently, L-thyroxine has been prepared in low yield by iodination of casein, followed by hydrolysis with sulphuric acid in the presence of butanol (Reineke and Turner, *J. Biol. Chem.*, 1943, **149**, 563), by oxidation of 3 : 5-di-iodo-L-tyrosine (Harington and Pitt Rivers, *Biochem. J.*, 1945, **39**, 157), and by the aerobic incubation of *N*-acetyl-3 : 5-di-iodo-L-tyrosine, followed by acid hydrolysis of the *N*-acetyl-L-thyroxine so formed (Pitt Rivers, *ibid.*, 1948, **43**, 223). However, no systematic synthesis of L-thyroxine from L-tyrosine has yet been described.

It appeared probable that the method of synthesis of DL-thyroxine described in Part III of this series (Borrows, Clayton, and Hems, this vol., p. S 199) would, with some modifications, be applicable to the preparation of L-thyroxine from L-tyrosine. (The more important steps in this synthesis are indicated in the right-hand part of the diagram.) Protection of the amino- and carboxy-groups by inclusion in a hydantoin ring, as in the synthesis of DL-thyroxine, was clearly unsuitable for the synthesis of L-thyroxine itself, since the conversion of 5-(3 : 5-di-iodo-4-*p*-hydroxyphenoxybenzyl)hydantoin (VII) into the amino-acid (XVI) would almost certainly result in racemisation. Thus, Dakin (*Amer. Chem. J.*, 1910, **44**, 48) has demonstrated the ready racemisation of hydantoins on treatment with alkali, and acid hydrolysis, which entails rather vigorous conditions, also results in an inactive product (Boyd, *Biochem. J.*, 1933, **27**, 1838). We have confirmed the latter observation : attempts to hydrolyse L-5-*p*-hydroxybenzylhydantoin (I) with hydrobromic acid gave the DL-hydantoin as the principal product, whilst the use of hydriodic acid and red phosphorus gave DL-tyrosine together with some of the racemised hydantoin.

However, since the reactions of the hydantoins were well known, it appeared desirable to repeat the synthesis of thyroxine, beginning with the hydantoin (I) derived from L-tyrosine, in order to determine the likelihood of racemisation in the earlier stages. In particular, the use of boiling pyridine in the conversion of a 2 : 6-dinitrophenol into a 2 : 6-dinitrodiphenyl ether seemed a possible source of trouble, since Abderhalden and Baumann (*Z. physiol. Chem.*, 1908, **55**, 412) have reported the racemisation of tryptophan on crystallisation from this solvent, though this result was not confirmed by Ellinger and Matsuoka (*ibid.*, 1914, **91**, 45). Some preliminary information was obtained on this point by boiling a solution of L-5-*p*-hydroxybenzylhydantoin in pyridine for 90 minutes, whereby no loss of optical activity resulted.

L-Tyrosine was converted into the hydantoin (I) by Dakin's method (*J. Biol. Chem.*, 1910, **8**, 25). Treatment of (I) with concentrated nitric acid at 25—30° gave L-5-(3-*nitro*-4-*hydroxybenzyl*)*hydantoin*, whereas nitration at 50° gave the required L-5-(3 : 5-*dinitro*-4-*hydroxybenzyl*)-*hydantoin* (II). Compound (II) was also prepared from 3 : 5-dinitro-L-tyrosine (III) (the preparation of which is described below) by reaction with sodium cyanate and ring-closure of the resulting hydantoic acid by means of hydrochloric acid. The rotations of (II) prepared by the two routes were identical. Compound (II) was converted into L-5-(3 : 5-*dinitro*-4-p-*methoxyphenoxybenzyl*)*hydantoin* (IV) by reaction with toluene-*p*-sulphonyl chloride in pyridine and treatment of the unisolated pyridinium toluene-*p*-sulphonate with *p*-methoxyphenol in the same solvent.

The rotation of (IV) obtained from different runs varied from 0° to −30°. The cause of this variation was traced to the crystallisation of (IV) from acetic acid in the presence of residual pyridine. Thus, on boiling (IV) ($[\alpha]_D$ −30·7°) with acetic acid containing 10% of pyridine, complete racemisation resulted within one hour. On the other hand, acetic acid alone and pyridine, alone or mixed with toluene-*p*-sulphonic acid, had little or no effect on the rotation. In the light of these observations, acetic acid was replaced by acetone in the isolation of (IV), but the rotation of different batches still varied from −23° to −36°.

The optical lability of compound (IV) was reflected in the results of its further reaction. Thus, in one instance, hydrogenation in acetic acid with palladised charcoal as catalyst gave the diamine (V); without purification, this was tetrazotised with a solution of sodium nitrite in concentrated sulphuric acid and thence converted into the *di-iodo*-compound (VI), which had $[\alpha]_D$ −11°. Repetition of this experiment under slightly different conditions led to a product

with $[\alpha]_D$ −2°; in this case, the intermediate diamine (V) was isolated as its monohydrochloride, and this also had a negligible rotation.

Compound (VI) ($[\alpha]_D$ −11°) was converted into 5-(3 : 5-di-iodo-4-*p*-hydroxyphenoxybenzyl)-hydantoin (VII) ($[\alpha]_D$ −12°) by treatment with a mixture of hydriodic and acetic acids, and (VII), in turn, was hydrogenolysed to 5-(4-*p*-hydroxyphenoxybenzyl)hydantoin (X), which had $[\alpha]_D$ −15·2°. A sample of (X) prepared from pure L-thyroxine (XVIII; $[\alpha]_D$ −5·4°) * by hydrogenolysis and treatment of the resulting L-thyronine (XVII) successively with sodium cyanate and hydrochloric acid had $[\alpha]_D$ −51°. Hence, it appeared that some 70% of racemisation had occurred between (II) and (X). That the reactions leading to the di-iodo-compound (VI) were responsible for this racemisation was indicated subsequently by the preparation of (VI) with $[\alpha]_D$ −50° from optically pure 3 : 5-*di-iodo*-4-p-*methoxyphenoxy*-N-*acetyl*-L-*phenylalanine ethyl ester* (XV) and of (VII) with $[\alpha]_D$ −43° from 3 : 5-di-iodo-L-thyronine (XVI). The preparation of (XV) and (XVI) is described below.

Greater success in retaining optical activity resulted from a slight modification of the route already described. Reaction of (II) with toluene-*p*-sulphonyl chloride in pyridine, followed by treatment with quinol, gave L-5-(3 : 5-*dinitro*-4-p-*hydroxyphenoxybenzyl*)*hydantoin* (VIII); this was converted *via* the diamine (IX) into L-5-(3 : 5-*di-iodo*-4-p-*hydroxyphenoxybenzyl*)*hydantoin* (VII); this had $[\alpha]_D$ −46° and on hydrogenolysis gave (X) with $[\alpha]_D$ −55°.

Although the results of the experiments on these hydantoins were far from consistent, yet they did indicate that none of the reactions leading to the required 2 : 6-di-iododiphenyl ethers was completely incompatible with the retention of optical activity and it seemed that greater success might result if means could be found of protecting the amino- and, if necessary, the carboxy-group of L-tyrosine by a system optically more stable than the hydantoin ring. The remainder of this paper describes such an approach, which has led to the preparation of L-thyroxine in high yield and without loss of optical activity.

Some difficulty has been encountered in the preparation of 3 : 5-dinitro-L-tyrosine (III). Two methods are recorded in the literature; one (Johnson and Kohmann, *J. Amer. Chem. Soc.*, 1915, **37**, 2164) involves a tedious isolation procedure and gives a poor yield, and the other (Waser, Labouchère, and Sommer, *Helv. Chim. Acta*, 1925, **8**, 773) involves the addition of L-tyrosine to a mixture of nitric and sulphuric acids kept below 0°, the product being isolated as its sodium salt. In our hands this second method has proved somewhat erratic; with certain modifications yields of 70% of (III) could often be obtained, but with small variations in the conditions the yield dropped to zero. More satisfactory results were obtained when nitric acid was added to a cooled suspension of L-tyrosine in sulphuric acid; on partial neutralisation, 3 : 5-dinitro-L-tyrosine was obtained in 86% yield, and no difficulty has been found in reproducing this yield.

Several attempts were made to prepare a diphenyl ether directly from (III) by its reaction with toluene-*p*-sulphonyl chloride in pyridine, followed by treatment with *p*-methoxyphenol in the same solvent, but no pure product could be isolated, and no more success attended the use of 3 : 5-*dinitro*-N-*toluene*-p-*sulphonyl*-DL-*tyrosine*.

Treatment of (III) in alkaline solution with acetic anhydride yielded the N-*acetyl* derivative (XI) in 80% yield. Compound (XI) failed to give a diphenyl ether on successive treatment with toluene-*p*-sulphonyl chloride and *p*-methoxyphenol in pyridine, and an attempt to use the preformed *toluene*-p-*sulphonyl* ester of (XI) for the diphenyl ether synthesis was equally unsuccessful.

It now seems very probable that the failure of these tyrosine derivatives to yield diphenyl ethers is associated with their free carboxylic acid groups. As described below, the *ethyl* ester (XII) of compound (XI) readily formed a diphenyl ether, and several other examples have been encountered of pairs of acids and esters, of which the ester, but not the acid, would undergo the diphenyl ether synthesis.

An attempt to esterify compound (XI) under Fischer–Speier conditions gave a high-melting solid, which we have been unable to identify. However, (XI) gave the ester (XII) in high yield on reaction with alcohol in the presence of toluene-*p*-sulphonic acid, water being removed azeotropically with chloroform. The *toluene*-p-*sulphonyl* ester of (XII), which could not be obtained crystalline, reacted with *p*-methoxyphenol in pyridine to give 3 : 5-*dinitro*-4-p-*methoxyphenoxy*-N-*acetyl*-L-*phenylalanine ethyl ester* (XIII). The diphenyl ether (XIII) was more conveniently prepared from (XII) by successive treatment with toluene-*p*-sulphonyl chloride and *p*-methoxyphenol in pyridine without isolation of the intermediate toluene-*p*-sulphonyl ester. In the same way, 3 : 5-*dinitro*-4-p-*acetoxyphenoxy*-N-*acetyl*-L-*phenylalanine*

* This sample of L-thyroxine was kindly supplied by Sir Charles Harington, F.R.S., to whom we express our thanks.

ethyl ester was prepared from *p*-acetoxyphenol. Hydrolysis of compound (XIII) with a mixture of hydrochloric and acetic acids gave the free *amino-acid*.

The conversion of (XIII) into 3 : 5-di-iodo-4-*p*-methoxyphenoxy-*N*-acetyl-L-phenylalanine ethyl ester (XV) was first effected without isolation of the intermediate diamine (XIV; R′ = H). The dinitro-compound (XIII) was reduced catalytically in acetic acid in the presence of palladised charcoal, and the crude amine, either in acetic acid or in phosphoric acid solution, was tetrazotised with a solution of sodium nitrite in concentrated sulphuric acid. Decomposition of the tetrazonium solution with an aqueous solution of iodine and sodium iodide gave (XV) in very poor yield. When the reduction of (XIII) was carried out in alcohol with either palladised charcoal or Raney nickel as catalyst, concentration of the filtered alcoholic solution gave the *diamine* (XIV; R′ = H) as a crystalline solid, which was moderately stable when dry, although in solution it was susceptible to aerial oxidation. The diamine was further characterised as its stable *diacetyl* derivative (XIV; R′ = Ac).

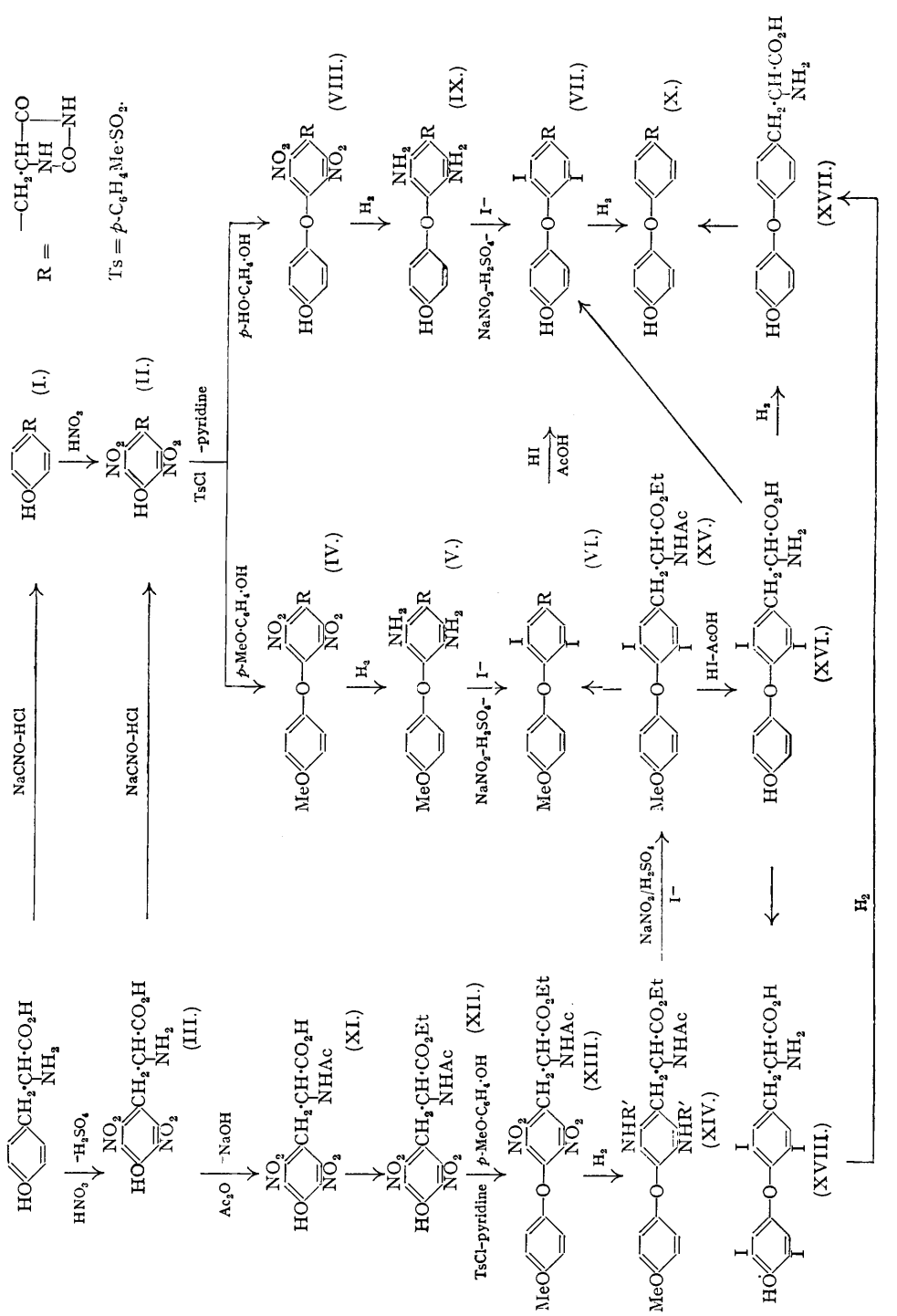
When the pure diamine was subjected to the tetrazotisation and Sandmeyer procedures, the yield of (XV) rose to about 45%. The conditions of both tetrazotisation and Sandmeyer reactions have been investigated in considerable detail in an effort to improve this yield, and some of the more significant observations may be quoted here. In agreement with the findings of Schoutissen (*J. Amer. Chem. Soc.*, 1933, **55**, 4531) we were unable to tetrazotise the diamine in concentrated sulphuric acid unless acetic or phosphoric acid was used as a diluent. Of these two acids, acetic was preferred owing to the greater ease with which the diamine dissolves; the yield was very much the same with either acid. The tetrazotisation was best carried out by the very slow addition of a solution of the diamine in a mixture of acetic and sulphuric acids to one of sodium nitrite in a mixture of the same acids kept between −2° and 0°. Under these conditions the tetrazonium solution did not become highly coloured, although under most other conditions deep purple colours developed, presumably owing to coupling with unreacted amine.

The replacement of the diazonium groups by iodine atoms was effected with a solution of iodine in aqueous sodium iodide. The yield dropped very considerably if free iodine was omitted. It was considered possible that the high concentration of acid might have an adverse effect upon the yield and it was, indeed, found that the yield of di-iodo-compound could be increased from 45% to 60% (based on diamine) by adding sodium hydroxide, sufficient to half-neutralise the sulphuric acid, concurrently with the addition of the tetrazonium solution. A further improvement in yield (to 77%) resulted when the reaction mixture, instead of being cooled below 30°, was allowed to warm to 50°, and it seemed likely that this might be due to the greater mobility at this temperature of the precipitated gum and hence its more intimate contact with the iodide solution. This supposition was tested by carrying out the reaction in the presence of chloroform, which should extract the di-iodo-compound as it is formed and so avoid the liberation of any solid or semi-solid matter during the reaction; under these conditions, the reaction was very rapid and the yield was over 80%. Further, neutralisation could be omitted without any reduction in yield.

Treatment of (XV) with a cold mixture of aqueous sodium hydroxide and alcohol caused hydrolysis of the ester group only and yielded 3 : 5-*di-iodo*-4-p-*methoxyphenoxy*-N-*acetyl*-L-*phenylalanine*, whereas a boiling mixture of hydrochloric and acetic acids gave the free *amino-acid*, which was used for the preparation of the hydantoin (VI) in an optically pure form.

Treatment of compound (XV) with mixtures of hydrobromic or hydriodic acid and glacial acetic acid gave 3 : 5-di-iodo-L-thyronine (XVI) in high yield. This reaction was also found to proceed satisfactorily with a mixture of only 2 mols. of hydriodic acid and an excess of hydrochloric and glacial acetic acids. The rotation quoted by Harington (*Biochem. J.*, 1928, **22**, 1949) for (XVI) in ammonia solution is only −1·3°, too low for the assessment of the optical purity of our product. However, on catalytic deiodination, our specimen of (XVI) gave rise to L-thyronine (XVII) with $[\alpha]_D$ +15·0°. Canzanelli, Harington, and Randall (*Biochem. J.*, 1934, **28**, 68) quote values of +12·2°, +13·9°, and +13·3° for material obtained from natural L-thyroxine, from 3 : 5-di-iodo-L-thyronine obtained by resolution, and from L-tyrosine by synthesis, respectively. It is of some interest that 3 : 5-di-iodo-L-thyronine (XVI) was found to have $[\alpha]_D$ +26° when dissolved in a mixture of hydrochloric acid and alcohol, whereas a solution in a mixture of sodium hydroxide and alcohol had no measurable rotation. This is in accordance with the rule that the rotations of L-amino-acids become more positive in the presence of acid.

3 : 5-Di-iodo-L-thyronine was converted into L-thyroxine (XVIII) by iodination with iodine in ethylamine solution. This novel method of iodination, which avoids the formation of explosive nitrogen iodides, is described in detail in Part VI (in the press). The L-thyroxine had


R = —CH₂·CH(NH)CO—NH·CO—
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Ac₂O
-NaOH
TsCl
-pyridine
p-MeO·C₆H₄·OH
p-HO·C₆H₄·OH
H₂
NaNO₂-H₂SO₄-
I⁻
HI
AcOH
HI-AcOH
NaNO₂/H₂SO₄
TsCl-pyridine

$[\alpha]_D$ −5·7° when dissolved in a mixture of ethanol and N-sodium hydroxide (2 : 1). This rotation is very similar to that quoted by Pitt Rivers (*loc. cit.*) for material prepared from 3 : 5-di-iodo-*N*-acetyl-L-tyrosine by aerobic incubation and is rather higher than the earlier values quoted in the literature (Harington and Salter, *loc. cit.*; Harington and Pitt Rivers, *loc. cit.*).

The overall yield of L-thyroxine, based on L-tyrosine, was 26%.

Experimental.

L-5-(3-*Nitro*-4-*hydroxybenzyl*)*hydantoin*.—L-5-*p*-Hydroxybenzylhydantoin (Dakin, *J. Biol. Chem.*, 1910, **8**, 25) (6 g.) was added in small portions to concentrated nitric acid (40 ml.) with vigorous stirring, the temperature being kept between 25° and 30° by addition of small pieces of solid carbon dioxide. The solution was stirred until there was a spontaneous drop of temperature (about 20 minutes after the completion of the addition) and was then poured into a large quantity of water. The flocculent yellow precipitate was filtered off and washed first with water and then with a small quantity of alcohol. After recrystallisation from alcohol (charcoal) the *hydantoin* was obtained as yellow prisms (5·8 g., 80%), m. p. 215—216° (Found: C, 48·3; H, 3·6; N, 16·9. $C_{10}H_9O_5N_3$ requires C, 47·8; H, 3·6; N, 16·7%); $[\alpha]_D^{18}$ −55·5° (*c*, 0·71 in acetone).

L-5-(3 : 5-*Dinitro*-4-*hydroxybenzyl*)*hydantoin* (II).—(*a*) *From* L-5-p-*hydroxybenzylhydantoin*. The hydantoin (10 g.) was added in portions to nitric acid (*d* 1·42; 100 ml.) which was stirred and kept at 45—50° during the addition, and for a further 2 hours. The solution was poured on ice and the yellow precipitate was filtered off and dried. Crystallisation from alcohol (charcoal) gave yellow needles, m. p. 249—250° (decomp.) (9·0 g., 63%) (Found: C, 40·95; H, 3·0; N, 18·9. $C_{10}H_8O_7N_4$ requires C, 40·5; H, 2·7; N, 18·9%); $[\alpha]_D^{18}$ −61·6° (*c*, 1·07 in acetone).

(*b*) *From* 3 : 5-*dinitro*-L-*tyrosine*. 3 : 5-Dinitro-L-tyrosine (for preparation, see below) (25 g.), sodium cyanate (17·5 g.), and water (125 ml.) were boiled under reflux for 15 minutes. A further quantity of sodium cyanate (10 g.) was added to the deep-red solution, which was then boiled under reflux for 30 minutes more. The solution was made acid to Congo-red by cautious addition of concentrated hydrochloric acid and after a short time the solid was filtered off. The moist solid was then boiled under reflux for 1 hour with 5N-hydrochloric acid (100 ml.). The mixture was allowed to cool and the solid (18 g., 66%) was filtered off. After crystallisation from alcohol, the material melted at 247—248° (decomp.) alone or mixed with a specimen prepared as described above (Found: N, 18·8%); $[\alpha]_D^{21}$ −62·0° (c, 1·00 in acetone).

L-5-(3 : 5-*Dinitro*-4-p-*methoxyphenoxybenzyl*)*hydantoin* (IV).—L-5-(3 : 5-Dinitro-4-hydroxybenzyl)-hydantoin (10·0 g.), toluene-*p*-sulphonyl chloride (7·2 g.), and dry pyridine (85 ml.) were boiled under reflux for 15 minutes. *p*-Methoxyphenol (12·5 g.) was added and the mixture was boiled under reflux for a further 90 minutes. The pyridine was removed by distillation under reduced pressure, and the residual gum was dissolved in acetone. On addition of water to the hot solution, a solid *product* separated (10·5 g., 77%), which melted at 236—236·5° after crystallisation from aqueous acetone (Found: C, 50·5; H, 3·4; N, 13·8. $C_{17}H_{14}O_8N_4$ requires C, 50·7; H, 3·5; N, 13·9%); $[\alpha]_D^{26}$ −29·6° (*c*, 0·98 in acetone). The rotations of the products obtained from several such experiments varied from −22·9° to −36·0°.

5-(3 : 5-*Di-iodo*-4-p-*methoxyphenoxybenzyl*)*hydantoin* (VI).—(*a*) L-5-(3 : 5-Dinitro-4-*p*-methoxyphenoxybenzyl)hydantoin ($[\alpha]_D^{19}$ −26·2°) (4·0 g.) in glacial acetic acid (70 ml.) was hydrogenated at 70°/85 atm. in the presence of palladised charcoal (10%; 1 g.). The catalyst was filtered off and the filtrate was added gradually to a cooled, stirred solution of sodium nitrite (1·6 g.) in concentrated sulphuric acid (50 ml.). When the addition was complete the solution was stirred for 30 minutes and then added to a 10% solution of iodine in concentrated aqueous potassium iodide (80 ml.). The mixture was left overnight and the solid material was filtered off and washed twice by suspension in hot sodium iodide solution. The resulting brown powder crystallised from glacial acetic acid (charcoal) to give the *di-iodo*-compound as a white solid, m. p. 212° (1·2 g., 19%), which contained solvent of crystallisation (Found: C, 37·1; H, 2·8; N, 4·65; I, 40·7. $C_{17}H_{14}O_4N_2I_2,C_2H_4O_2$ requires C, 36·6; H, 2·9; N, 4·5; I, 40·7%); $[\alpha]_D^{19}$ −11·0° (*c*, 0·988 in acetone).

(*b*) The dinitro-compound ($[\alpha]_D$ −27°; 11·7 g.) in glacial acetic acid (300 ml.) was hydrogenated at room temperature and 100 atm. in the presence of palladised charcoal (10%; 1 g.). The catalyst was filtered off, and the filtrate evaporated under reduced pressure from a bath at 50°. The residue was dissolved in acetone and treated with a slight excess of methyl-alcoholic hydrogen chloride. The acetone was decanted from the semi-solid hydrochloride which was crystallised from a mixture of alcohol with either chloroform or ethyl acetate. 5-(3 : 5-Diamino-4-*p*-methoxyphenoxybenzyl)hydantoin monohydrochloride (7·7 g., 70%) melted at 246° (decomp.) alone or mixed with a specimen of the DL-compound and had no measurable rotation (Found: N, 14·5; Cl, 9·3. Calc. for $C_{17}H_{19}O_4N_4Cl$: N, 14·8; Cl, 9·4%). Borrows, Clayton, and Hems (this vol., p. S 199) give m. p. 245° (decomp.) for the DL-compound.

The hydrochloride (2·0 g.) was added to a cooled, stirred mixture of acetic acid (20 ml.) and sulphuric acid (20 ml.). The solution was added during 2 hours to a cooled (−2° to 0°), well-stirred solution of sodium nitrite (0·9 g.) in sulphuric acid (12 ml.), previously diluted below 0° with acetic acid (12 ml.). After a further hour, the orange-coloured tetrazonium solution was added to a solution of sodium iodide (5 g.) and iodine (6 g.) in water (100 ml.), which was stirred and kept below 25°. The mixture was stirred overnight and then filtered. The black solid was suspended in sodium hydrogen sulphite solution and treated with sulphur dioxide to remove free iodine. The resulting pale yellow solid (2·4 g., 81%), crystallised from aqueous acetone, melted at 211—213°, alone or mixed with a specimen of the DL-compound (Borrows, Clayton, and Hems, *loc. cit.*, give m. p. 213—214° for the DL-compound); $[\alpha]_D$ −2° (*c*, 1·0 in acetone).

A preparation of the optically pure L-compound from the corresponding amino-acid is described on p. 3432.

Demethylation of 5-(3 : 5-*Di-iodo*-4-p-*methoxyphenoxybenzyl*)*hydantoin and Conversion of the Product* (VII) *into* 5-(4-p-*Hydroxyphenoxybenzyl*)*hydantoin* (X).—The foregoing methoxy-compound ($[\alpha]_D$ −11·0°) (2·0 g.) was boiled under reflux for 30 minutes with a mixture of hydriodic acid (*d* 1·7; 6 ml.) and glacial acetic acid (6 ml.). The mixture was left overnight in the refrigerator and the solid material was filtered off and washed with a little glacial acetic acid. After crystallisation from aqueous alcohol the material was obtained as a white powder (1·67 g., 85%), m. p. 247° (Found: N, 5·2. Calc. for $C_{16}H_{12}O_4N_2I_2$: N, 5·1%); $[\alpha]_D^{20}$ −11·8° (*c*, 0·51 in alcohol).

The di-iodo-compound (0·5 g.) in N-sodium hydroxide (20 ml.) was hydrogenolysed in the presence of palladised calcium carbonate (1%; 2 g.). After removal of the catalyst the boiling solution was treated with an excess of dilute hydrochloric acid to yield 5-(4-p-*hydroxyphenoxybenzyl*)*hydantoin* (0·23 g.), m. p. 256—257° (decomp.) (Found: N, 9·4. $C_{16}H_{14}O_4N_2$ requires N, 9·4%), $[\alpha]_D$ −15·2° (*c*, 0·462 in ethanol).

L-5-(3 : 5-*Dinitro*-4-p-*hydroxyphenoxybenzyl*)*hydantoin* (VIII).—A mixture of L-5-(3 : 5-dinitro-4-hydroxybenzyl)hydantoin (1·0 g.), toluene-*p*-sulphonyl chloride (0·74 g.), and dry pyridine (10 ml.) was boiled under reflux for 15 minutes. Quinol (1·5 g.) was added and the mixture was boiled under reflux for 90 minutes. The pyridine was removed under reduced pressure, and the residual oil dissolved in warm acetone which was then diluted with a large volume of water. The precipitated solid was filtered off, dried, and extracted with cold acetone. A small quantity of undissolved solid was removed and the filtrate was evaporated to small bulk and diluted with water. The resulting solid (0·7 g.) was again extracted with cold acetone, and the hot solution diluted with water to the point of precipitation. The small quantity of solid which separated on cooling was filtered off and the filtrate was boiled till solid began to separate. The *compound*, which was obtained as fine, yellow needles, melted at 230° (decomp.), unchanged by further crystallisation from aqueous acetone (Found: C, 49·7; H, 3·6; N, 14·3. $C_{16}H_{12}O_8N_4$ requires C, 49·5; H, 3·1; N, 14·4%); $[\alpha]_D^{20}$ −38·1° (*c*, 0·971 in dioxan).

L-5-(3 : 5-*Di-iodo*-4-p-*hydroxyphenoxybenzyl*)*hydantoin* (VII).—The dinitro-compound (3·0 g.) in glacial acetic acid (70 ml.) was hydrogenated at 80°/75 atm. in the presence of palladised charcoal. The solution was filtered when hot and then allowed to cool, whereupon the diamine (0·98 g.) separated. A second fraction (1·07 g.) was obtained by concentration of the mother-liquors.

The diamine (0·98 g.) was dissolved in phosphoric acid (25 ml.), and the solution added dropwise to a cooled, stirred solution of sodium nitrite (0·35 g.) in concentrated sulphuric acid (12 ml.). The solution was stirred and cooled for 30 minutes after the addition was complete and was then run into a stirred solution of iodine (1·25 g.) and sodium iodide (1·5 g.) in water (25 ml.). After storage overnight, the solid was filtered off, washed free from iodine by repeated treatment with boiling sodium iodide solution, and dried. L-5-(3 : 5-Di-iodo-4-*p*-hydroxyphenoxybenzyl)hydantoin (0·67 g.) was obtained as a light brown powder, m. p. 255°, $[\alpha]_D^{20}$ −46° (*c*, 0·5 in dioxan). This material was converted into L-5-(4-*p*-hydroxyphenoxybenzyl)hydantoin without further purification. A preparation of the same compound from 3 : 5-di-iodo-L-thyronine is described on p. 3432.

L-5-(4-p-*Hydroxyphenoxybenzyl*)*hydantoin* (X).—(*a*) *From* L-5-(3 : 5-*di-iodo*-4-p-*hydroxyphenoxybenzyl*)*hydantoin*. The hydantoin ($[\alpha]_D$ −46°) (0·94 g.) in a mixture of 0·2N-sodium hydroxide (40 ml.) and alcohol (10 ml.) was hydrogenolysed at room temperature and pressure in the presence of palladised calcium carbonate (1%; 1 g.). After removal of the catalyst the solution was acidified with hydrochloric acid and the *hydantoin* (0·48 g.) was filtered off. After crystallisation from aqueous acetic acid it melted at 252° (Found: N, 9·4. $C_{16}H_{14}O_4N_2$ requires N, 9·4%); $[\alpha]_D^{20}$ −54·6° (*c*, 0·861 in alcohol).

(*b*) *From* L-*thyroxine* (*see also p.* 3433). L-Thyroxine ($[\alpha]_D$ −5·4°) (0·4 g.) in 0·2N-sodium hydroxide (26 ml.) was hydrogenolysed at room temperature and pressure in the presence of palladised calcium carbonate (1%; 1 g.). The catalyst was filtered off and the filtrate was brought to pH *ca.* 8·5 by addition of acetic acid. Sodium cyanate (0·4 g.) was added, and the mixture boiled under reflux for 60 minutes. Concentrated hydrochloric acid (20 ml.) was then added, and the mixture boiled for a further 30 minutes. After evaporation to small bulk the mixture was filtered and the solid (0·12 g.) was crystallised from aqueous acetic acid, being obtained as small needles, m. p. 252° (Found: N, 9·2%), $[\alpha]_D^{20}$ −50·7° (*c*, 0·73 in alcohol).

3 : 5-*Dinitro*-L-*tyrosine* (III).—L-Tyrosine (105 g.) was added in portions to concentrated sulphuric acid (450 ml.) which was stirred and kept at 10° by external cooling. When the addition was complete the mixture was cooled to −5° and stirred while nitric acid (*d* 1·42; 85·5 ml.) was added dropwise, the addition taking some 90 minutes. The solution was then stirred at 0° for 15 minutes and poured on crushed ice (2 kg.). A solution of sodium hydroxide (400 g.) in water (1 l.) was added cautiously and the mixture was then cooled, giving yellow crystals of 3 : 5-dinitro-L-tyrosine (137 g., 87%), m. p. 230—232° (decomp.) (Found, after drying at 120°: C, 39·6; H, 3·7. Calc. for $C_9H_9O_7N_3$: C, 39·8; H, 3·3%) (Waser, Labouchère, and Sommer, *loc. cit.*, state that the compound explodes at 230° on rapid heating).

3 : 5-*Dinitro*-N-*toluene*-p-*sulphonyl*-DL-*tyrosine*.—3 : 5-Dinitro-DL-tyrosine (2·72 g.) was dissolved in 2N-sodium hydroxide (20 ml.) and the solution was shaken overnight with toluene-*p*-sulphonyl chloride (7·6 g.) in ether (40 ml.). The aqueous layer was separated, extracted once with ether, and acidified with 2N-hydrochloric acid. The *dinitro*-compound (1·86 g., 44%) was crystallised from glacial acetic acid and obtained as fine crystals, m. p. 221° (Found: C, 45·7; H, 3·6; N, 9·6; S, 7·5. $C_{16}H_{15}O_9N_3S$ requires C, 45·2; H, 3·6; N, 9·9; S, 7·5%). The compound was shown to be the *N*-toluene-*p*-sulphonyl derivative by the fact that it dissolved in alkali to give a deep-red solution, indicating that the phenolic group was free.

3 : 5-*Dinitro*-N-*acetyl*-L-*tyrosine* (XI).—A solution of 3 : 5-dinitro-L-tyrosine (100 g.) in 2N-sodium hydroxide (800 ml.) was treated with acetic anhydride (51·2 ml.), the solution being stirred and the temperature kept below 20°. When the addition was complete the solution was stirred at room temperature for 1 hour and then heated to 40° for 30 minutes in order to decompose excess of acetic anhydride. Hydrochloric acid (5N.) was added dropwise till the solution was acid to Congo-red and the *acetyl* compound (95 g., 82%) was filtered off. After crystallisation from alcohol or ethyl acetate, it melted at 189—190° (Found: C, 42·5, H, 3·5; N, 13·3. $C_{11}H_{11}O_8N_3$ requires C, 42·2; H, 3·5; N, 13·4%); $[\alpha]_D^8$ +12·2° (*c*, 1·15 in dioxan).

3 : 5-*Dinitro*-4-*toluene*-p-*sulphonyloxy*-N-*acetyl*-L-*phenylalanine*.—3 : 5-Dinitro-*N*-acetyl-L-tyrosine (1 g.) in 0·5N-sodium carbonate (20 ml.) was shaken for 20 hours with a solution of toluene-*p*-sulphonyl chloride (1·5 g.) in ether (20 ml.). The aqueous layer was separated and acidified with 2N-hydrochloric acid; the pale yellow precipitate of *toluene*-p-*sulphonyl* derivative crystallised from aqueous alcohol in small white crystals (0·2 g.), m. p. 178° (Found: C, 46·4; H, 4·0; N, 9·3. $C_{18}H_{17}O_{10}N_3S$ requires C, 46·3; H, 3·7; N, 9·0%); $[\alpha]_D^{18}$ −5·5° (*c*, 0·72 in dioxan).

3 : 5-*Dinitro*-N-*acetyl*-L-*tyrosine Ethyl Ester* (XII).—3 : 5-Dinitro-N-acetyl-L-tyrosine (32 g.), toluene-*p*-sulphonic acid (3 g.), alcohol (21 ml.), and chloroform (1 l.) were boiled under reflux in a flask fitted with an automatic water separator. When no more water was carried over in the chloroform (about 8 hours), the solution was cooled and extracted twice with 2N-sodium carbonate solution. The aqueous extract was acidified to Congo-red with 2N-hydrochloric acid, giving a thick brown oil, which solidified when it was gently warmed and scratched. After crystallisation from hot water or from ethyl acetate—petroleum (b. p. 60—80°) the *ethyl* ester (30 g., 86%) melted at 120—121° (Found: C, 46·1; H, 4·7; N, 12·4. $C_{13}H_{15}O_8N_3$ requires C, 45·8; H, 4·4; N, 12·3%); $[\alpha]_D^{23}$ −6·75° (*c*, 6·2 in dioxan).

3 : 5-*Dinitro*-4-*toluene*-p-*sulphonyloxy*-N-*acetyl*-L-*phenylalanine Ethyl Ester*.—3 : 5-Dinitro-*N*-acetyl-L-tyrosine ethyl ester (3·41 g.), toluene-*p*-sulphonyl chloride (2·0 g.), N-sodium hydroxide (10 ml.), and acetone (50 ml.) were boiled under reflux for an hour, during which the red colour disappeared completely. Most of the acetone was distilled off, giving an oil which could not be induced to solidify. The oil was extracted into chloroform, and the extract washed with 2N-sodium carbonate, then with water, and dried ($CaCl_2$). The chloroform extract was evaporated to dryness and the residual gum was triturated with light petroleum, giving a glass, which could not be crystallised from the normal solvents. Purification was effected by heating at 100°/0·03 mm. in order to remove unchanged toluene-*p*-sulphonyl chloride. The residual *toluene*-p-*sulphonyl* ester was triturated with petroleum but again it was impossible to crystallise the glassy product (Found: N, 7·9; S, 6·4. $C_{20}H_{21}O_{10}N_3S$ requires N, 8·5; S, 6·5%).

3 : 5-*Dinitro*-4-p-*methoxyphenoxy*-N-*acetyl*-L-*phenylalanine Ethyl Ester* (XIII).—(a) *From* 3 : 5-*dinitro*-4-*toluene*-p-*sulphonyloxy*-N-*acetyl*-L-*phenylalanine ethyl ester*. The toluene-*p*-sulphonyl ester (0·8 g.), *p*-methoxyphenol (0·6 g.), and pyridine (4 ml.) were boiled under reflux for 1 hour. The solution was poured into an excess of 2N-hydrochloric acid, and the deposited oil extracted into chloroform. The extract was washed successively with 2N-hydrochloric acid, 2N-sodium hydroxide, and water. The dried extract was evaporated, and the residue crystallised from alcohol. The diphenyl ether (0·41 g.) crystallised from benzene–petroleum (b. p. 60—80°) to give material melting at 104—106°.

(b) *Directly from* 3 : 5-*dinitro*-N-*acetyl*-L-*tyrosine ethyl ester*. A solution of the ester (96 g.) and toluene-*p*-sulphonyl chloride (60 g.) in dry pyridine (80 ml.) was heated on the steam-bath for 30 minutes. *p*-Methoxyphenol (102·6 g.) was added, and the solution boiled under reflux for one hour. The pyridine was removed under reduced pressure and the residue was taken up in chloroform and extracted thoroughly with 2N-hydrochloric acid, 2N-sodium hydroxide, and water. The chloroform solution was then evaporated to dryness. The residue, on crystallisation from ethanol, gave 3 : 5-*dinitro*-4-p-*methoxyphenoxy*-N-*acetyl*-L-*phenylalanine ethyl ester* (85 g., 68%), m. p. 109—110° (Found: C, 53·4; H, 4·7; N, 9·4. $C_{20}H_{21}O_9N_3$ requires C, 53·7; H, 4·7; N, 9·4%); $[\alpha]_D^{27}$ −8·2° (*c*, 0·98 in dioxan).

3 : 5-*Dinitro*-4-p-*methoxyphenoxy*-L-*phenylalanine*.—3 : 5-Dinitro-4-*p*-methoxyphenoxy-*N*-acetyl-L-phenylalanine ethyl ester (5 g.) was boiled under reflux for 2 hours with a mixture of concentrated hydrochloric acid (25 ml.) and glacial acetic acid (25 ml.). On cooling, a solid separated, which was filtered off, washed well with water, dried, and crystallised from aqueous acetic acid. The *amino-acid* (3·7 g., 88%) melted at 247—248° (decomp.) (Found: C, 51·1; H, 4·2; N, 11·15. $C_{16}H_{15}O_8N_3$ requires C, 50·95; H, 4·0; N, 11·1%); $[\alpha]_D^{23}$ +25·4° (*c*, 1·54 in equal volumes of N-hydrochloric acid and alcohol).

3 : 5-*Dinitro*-4-p-*acetoxyphenoxy*-N-*acetyl*-L-*phenylalanine Ethyl Ester*.—3 : 5-Dinitro-*N*-acetyl-L-tyrosine ethyl ester (1·37 g.), toluene-*p*-sulphonyl chloride (0·75 g.), and pyridine (20 ml.) were heated on a steam-bath to 60—70°. *p*-Acetoxyphenol (1·52 g.) was added, and the solution boiled under reflux for one hour. Pyridine was removed under reduced pressure, and the residual oil extracted with boiling ethyl acetate. The solution was decanted from a little insoluble material and evaporated to dryness. The residual gum was dissolved in acetone and passed through a column of alumina. On removal of the acetone the gum rapidly crystallised. The solid *product* (1·05 g., 55%), crystallised from benzene, melted at 114—117° (Found: C, 52·7; H, 4·4; N, 8·8. $C_{21}H_{21}O_{10}N_3$ requires C, 53·0; H, 4·4; N, 8·8%).

3 : 5-*Diamino*-4-p-*methoxyphenoxy*-N-*acetyl*-L-*phenylalanine Ethyl Ester* (XIV; R′ = H).—3 : 5-Dinitro-4-*p*-methoxyphenoxy-*N*-acetyl-L-phenylalanine ethyl ester (106 g.) in methanol (1 l.) was hydrogenated at room temperature and 70 atm. in the presence of palladised charcoal (10%; 6 g.). During all the subsequent manipulations air was excluded by means of carbon dioxide. While warm (50°) the solution was filtered, the catalyst was washed with warm methanol (200 ml.), and the combined filtrate and washings were evaporated to dryness under reduced pressure, the temperature being kept as low as possible. The residual gum was dissolved in warm ethanol (*ca.* 100 ml.). On cooling, the *diamine* (87 g., 95%) separated as white crystals. Material obtained from the first runs melted at 73—75°, resolidified at a higher temperature, and finally melted at 135—136°. On storage, the crystals changed spontaneously into the high-melting form and later batches have consisted only of this form (Found: C, 61·8; H, 6·4; N, 10·65. $C_{20}H_{25}O_5N_3$ requires C, 62·0; H, 6·45; N, 10·85%); $[\alpha]_D^{26}$ +42·4° (*c*, 0·94 in dioxan).

Diacetyl derivative (XIV; R′ = Ac). (*a*) The crude diamine, obtained by evaporation of the hydrogenation solution, was dissolved in boiling acetic anhydride. On storage at room temperature overnight, crystals separated, which were filtered off and washed with dilute sodium hydroxide solution and water. The *diacetamido*-compound crystallised from alcohol as colourless needles, m. p. 226—227° (Found: C, 61·4; H, 6·35; N, 8·7. $C_{24}H_{29}O_7N_3$ requires C, 61·15; H, 6·2; N, 8·9%); $[\alpha]_D^{18}$ +72·5° (*c*, 1·01 in chloroform).

(*b*) The crystalline diamine (1 g.) was left for 12 hours at room temperature in dry pyridine (10 ml.) containing acetic anhydride (1·2 ml.). The solid which separated was washed with dilute hydrochloric acid, dilute sodium hydroxide, and water. After crystallisation from alcohol the compound (0·8 g., 66%) melted at 226°; $[\alpha]_D^{18}$ +72° (*c*, 1·02 in chloroform).

3 : 5-*Di-iodo*-4-p-*methoxyphenoxy*-N-*acetyl*-L-*phenylalanine Ethyl Ester* (XV).—A solution of 3 : 5-diamino-4-*p*-methoxyphenoxy-*N*-acetyl-L-phenylalanine ethyl ester (40 g.) in acetic acid (80 ml.) was added to concentrated sulphuric acid (40 ml.) with stirring, the temperature being kept between 10° and 20°. This solution was added dropwise during about 2 hours to a stirred and cooled (−2° to 0°) solution, prepared by cautious dilution with glacial acetic acid (250 ml.) at about 0° of a solution of sodium nitrite (17·5 g.) in concentrated sulphuric acid (125 ml.). When the addition of the diamine solution was complete, the orange-coloured solution was stirred for one hour at 0° and was then added fairly rapidly from a cooled dropping-funnel to a well-stirred solution of sodium iodide (80 g.), iodine (67 g.), and urea (10 g.) in water (1300 ml.) covering a layer of chloroform (250 ml.). No attempt was made to cool the mixture, the temperature of which rose to about 40°. Stirring was continued for an hour after the addition had been completed and the chloroform layer was then separated. The aqueous layer, together with some undissolved iodine, was washed twice with chloroform, and the combined chloroform solutions were washed with water. Free iodine was removed from the chloroform solution by covering it with an aqueous solution of sodium sulphite (100 g.) and passing in sulphur dioxide. The solution was again washed with water and evaporated to dryness, leaving a solid residue. After crystallisation from alcohol, the *di-iodo*-compound (51·5 g., 82%) melted at 143—144° (Found: C, 39·4; H, 3·5; N, 2·3; I, 41·8. $C_{20}H_{21}O_5NI_2$ requires C, 39·4; H, 3·5; N, 2·3; I, 41·7%); $[\alpha]_D^{23}$ +30·8° (*c*, 6·04 in dioxan).

3 : 5-*Di-iodo*-4-p-*methoxyphenoxy*-N-*acetyl*-L-*phenylalanine*.—3 : 5-Di-iodo-4-*p*-methoxyphenoxy-*N*-acetyl-L-phenylalanine ethyl ester (0·5 g.) was dissolved in a mixture of N-sodium hydroxide (7 ml.) and alcohol (7 ml.). The solution was left at room temperature for 30 minutes and was then acidified to Congo-red with hydrochloric acid; the solid *acid* was filtered off and crystallised from aqueous alcohol, being obtained as colourless crystals, m. p. 196—197° (Found: N, 2·3; I, 42·5; OMe, 5·4. $C_{18}H_{17}O_5NI_2$ requires N, 2·4; I, 43·7; OMe, 5·3%); $[\alpha]_D^{20}$ +8° (*c*, 0·875 in acetone).

In order to show that no racemization had occurred during hydrolysis, the compound was re-esterified by treatment with alcohol in the presence of toluene-*p*-sulphonic acid, boiling chloroform being used to entrain the water formed. The ester so formed melted at 143° and its rotation was not significantly different from that of an authentic specimen.

3 : 5-*Di-iodo*-4-p-*methoxyphenoxy*-L-*phenylalanine*.—3 : 5-Di-iodo-4-*p*-methoxyphenoxy-*N*-acetyl-L-phenylalanine ethyl ester (2 g.) was boiled under reflux for 2 hours with a mixture of concentrated hydrochloric acid (10 ml.) and glacial acetic acid (10 ml.). The mixture was diluted with water and, after some time, the solid was filtered off, washed with water, and dried. After crystallisation from aqueous pyridine the *amino-acid* was obtained as fine white needles, m. p. 241° (decomp.) (Found: N, 2·7; I, 46·5; OMe, 5·5. $C_{16}H_{15}O_4NI_2$ requires N, 2·6; I, 47·1; OMe, 5·8%); $[\alpha]_D^{26}$ +19·4° (*c*, 1·02 in equal volumes of N-HCl and alcohol), $[\alpha]_D^{22}$ −6·7° (*c*, 1·05 in equal volumes of N-NaOH and alcohol); the yield was quantitative.

L-5-(3 : 5-*Di-iodo*-4-p-*methoxyphenoxybenzyl*)*hydantoin* (VI).—3 : 5-Di-iodo-4-*p*-methoxyphenoxy-L-phenylalanine (2·0 g.), sodium cyanate (2·0 g.), and water (40 ml.) were boiled together under reflux for 1 hour. Since much solid remained undissolved, further quantities of sodium cyanate (2·0 g.) and alcohol (40 ml.) were added and the mixture was refluxed for a further 2 hours. After cooling, the mixture was carefully acidified with hydrochloric acid and filtered. The solid was boiled under reflux for 90 minutes with a mixture of 5N-hydrochloric acid (20 ml.) and alcohol (20 ml.). The mixture was left overnight in the refrigerator and filtered. The solid was extracted with acetone to remove a little insoluble material, and the product obtained by dilution of the filtrate with water was further crystallised from aqueous acetone to yield the *hydantoin* (1·1 g.), m. p. 243—245° (Found: C, 36·3; H, 2·7; N, 4·65; I, 45·4. $C_{17}H_{14}O_4N_2I_2$ requires C, 36·2; H, 2·5; N, 5·0; I, 45·0%); $[\alpha]_D^{25}$ −50·2° (*c*, 1·045 in acetone).

3 : 5-*Di-iodo*-L-*thyronine* (XVI).—A mixture of 3 : 5-di-iodo-4-*p*-methoxyphenoxy-*N*-acetyl-L-phenylalanine ethyl ester (10 g.), hydriodic acid (57%, 20 ml.), and glacial acetic acid (20 ml.) was boiled under reflux for 4 hours. The solution was evaporated to dryness under reduced pressure and the residue was dissolved in hot ethyl alcohol (70 ml.). To the boiling alcoholic solution was added a hot solution of sodium acetate (trihydrate; 15 g.) and sodium metabisulphite (0·5 g.) in water (50 ml.). After being left in the refrigerator for a short time, the white crystalline precipitate was filtered off, washed with water and alcohol, and dried. The material was purified by dissolving it in alcohol containing a little concentrated hydrochloric acid, decolorising the solution with charcoal, diluting with water, and precipitating the amino-acid by the addition of boiling sodium acetate solution. The product (7·9 g., 90%) melted with decomposition at 255° (Found: C, 34·0; H, 2·8; N, 2·7. Calc. for $C_{15}H_{13}O_4NI_2$: C, 34·3; H, 2·5; N, 2·7%); $[\alpha]_D^{22}$ +26° (*c*, 1·06 in a 1 : 2 mixture of N-hydrochloric acid and alcohol). Harington (*Biochem. J.*, 1928, **22**, 1429) gives m. p. 256° (decomp.).

A similar yield of 3 : 5-di-iodo-L-thyronine resulted when 3 : 5-di-iodo-4-*p*-methoxyphenoxy-*N*-acetyl-L-phenylalanine ethyl ester (5 g.) was boiled under reflux for 18 hours with either a mixture of hydrobromic acid (47%; 7·5 ml.) and glacial acetic acid (7·5 ml.) or a mixture of hydriodic acid (57%; 4 ml.), concentrated hydrochloric acid (15 ml.), and glacial acetic acid (11 ml.). When the latter mixture was employed, a solid separated on cooling and this was filtered off after water (35 ml.) had been added and the mixture left for a short time. This material was purified by precipitation with sodium acetate from its solution in alcohol and hydrochloric acid.

L-5-(3 : 5-*Di-iodo*-4-p-*hydroxyphenoxybenzyl*)*hydantoin* (VII).—3 : 5-Di-iodo-L-thyronine (0·6 g.) was treated with sodium cyanate (0·6 g.) in boiling water (20 ml.) until a clear solution was obtained. The brown solution was decolorised with charcoal, concentrated hydrochloric acid (6 ml.) added, and the mixture then boiled under reflux for 30 minutes. The solid *hydantoin* was filtered off and crystallised from aqueous acetic acid; m. p. 255° (Found: N, 5·3. $C_{16}H_{12}O_4N_2I_2$ requires N, 5·1%); $[\alpha]_D^{19}$ −43° (*c*, 1·18 in dioxan).

L-*Thyronine* (XVII).—3 : 5-Di-iodo-L-thyronine was hydrogenolysed as described by Canzanelli, Harington, and Randall (*Biochem. J.*, 1934, **28**, 68). The L-thyronine so obtained melted at 255° (decomp.) (Found: C, 66·1; H, 5·7; N, 5·0. Calc. for $C_{15}H_{15}O_4N$: C, 65·9; H, 5·5; N, 5·1%); $[\alpha]_D^{18}$ +15·0° (*c*, 1·36 in equal volumes of N-hydrochloric acid and alcohol). Canzanelli, Harington, and

Randall (*loc. cit.*) give m. p. 252° (decomp.) and $[\alpha]_{5461}$ +13·9° for material prepared by this method. The hydantoin (X), prepared from this L-thyronine in the usual way, melted at 253—254° after crystallisation from aqueous acetic acid and had $[\alpha]_D^{20}$ −54·7° (*c*, 0·74 in alcohol) (Found: N, 9·5. Calc. for $C_{16}H_{14}O_4N_2$: N, 9·4%).

L-*Thyroxine* (XVIII).—To a stirred solution of 3 : 5-di-iodo-L-thyronine (22 g.) in aqueous ethylamine (33%; 220 ml.) a 1·9N-solution (88 ml.) of iodine in concentrated aqueous potassium iodide was added dropwise. Towards the end of the addition, the ethylamine salt of L-thyroxine began to separate. After all the iodine solution had been added, the mixture was stirred for a few minutes and hydrochloric acid (16%) was then added till the pH of the solution was 4—5. After 2 hours, the precipitated thyroxine was filtered off, washed with water, and purified by dissolving it in a mixture of ethyl alcohol (250 ml.) and 2N-sodium hydroxide (100 ml.), and adding hot 2N-hydrochloric acid to the boiling solution till the pH reached 4—5. The mixture was left in the refrigerator for a few hours and the L-thyroxine was then filtered off and dried (yield 29 g., 89%); m. p. 233—235° (decomp.) (Found: C, 23·4; H, 1·4; N, 1·6; I, 65·3. Calc. for $C_{15}H_{11}O_4NI_4$: C, 23·2; H, 1·4; N, 1·8; I, 65·3%); $[\alpha]_D^{20}$ −5·7° (*c*, 2·2 in a 1 : 2 mixture of N-sodium hydroxide and ethyl alcohol). Pitt Rivers (*Biochem. J.*, 1948, **43**, 223) gives m. p. 232° (decomp.) and $[\alpha]_D^{21}$ −5·4°.

L-*Thyroxine mono-sodium salt.* L-Thyroxine (10 g.) was added carefully to boiling 2N-sodium carbonate (240 ml.). The solution deposited the *mono-sodium* salt on cooling as a white or pale yellow solid, which was filtered off, washed with water and alcohol, and dried in a desiccator; yield 10·5 g., 93%. This material lost 10% of its weight on drying at 100° under reduced pressure, indicating that it contained 5 molecules of water of crystallisation (Found, on material dried at 100°: I, 64·0. $C_{15}H_{10}O_4NI_4Na$ requires I, 63·6%).

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