297. *The Synthesis of Thyroxine and Related Substances. Part XIII.* Some Further Analogues of Thyroxine.*

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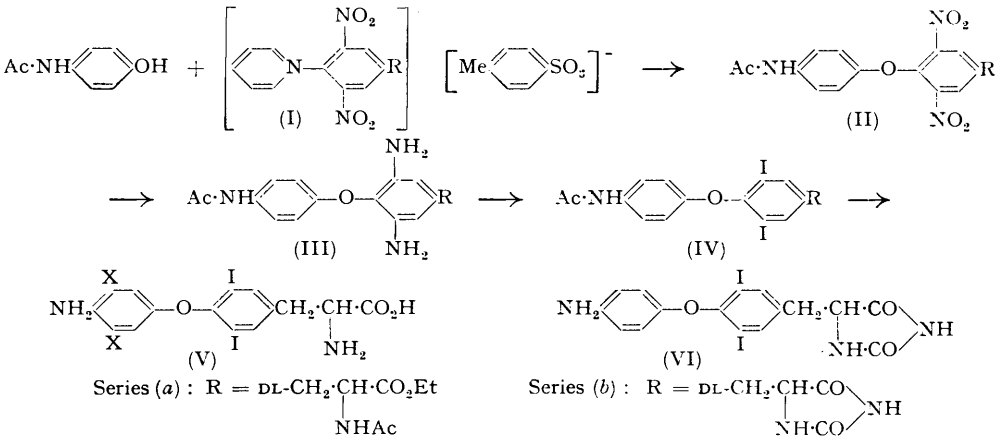
The preparation is described of the two "deoxythyroxines," 4-(3 : 5-di-iodophenoxy)-3 : 5-di-iodo-DL-phenylalanine (XV; R = R′ = H) and 4-(4-hydroxy-3 : 5-di-iodophenyl)-3 : 5-di-iodo-DL-phenylalanine (XXI), and of 3 : 5-di-iodo-3′ : 5′-dimethyl-DL-thyronine (XXVII). An attempt to prepare the 4′-amino-analogue of thyroxine failed, as 4-*p*-aminophenoxy-3 : 5-di-iodo-DL-phenylalanine (V; X = H) could not be iodinated. Some experiments leading towards the synthesis of the 3 : 5-dimethyl- and the 4′-mercapto-analogues of thyroxine are described. A new method of preparation of the known 2′ : 6′-di-iodothyronine is reported.

Several analogues of thyroxine have been reported that differ from the hormone in the nature of the side chain, in having the iodine atoms replaced by other halogen atoms, or in having the ether-oxygen atom replaced by sulphur. These minor variations have led so far to compounds with thyroxine-like activity rather than to thyroxine antagonists. It appears possible, nevertheless, that a close structural analogue might be found that, by competition with the hormone at the site of action, would act as an antagonist, and this paper describes the preparation of further analogues, of which two had the desired activity to a slight extent.



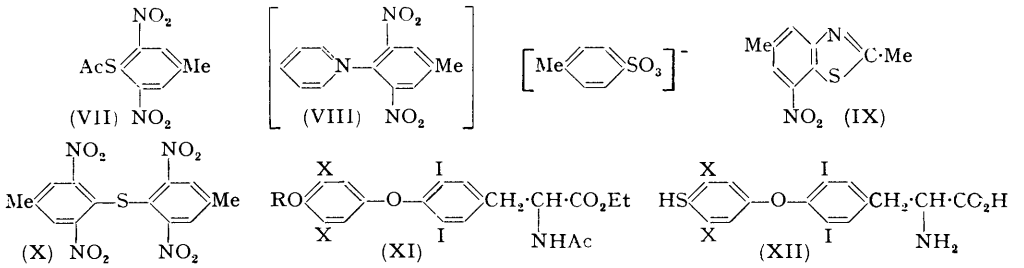
First, the preparation was attempted of compound (V; X = I) in which an amino-group replaces the phenolic hydroxyl of thyroxine. Reaction of aminophenols with quaternary toluene-*p*-sulphonates derived from 2 : 6-dinitrophenols is known to involve the amino- rather than the phenolic group (Cookson, Part XI, *J.*, 1953, 643). However, *p*-acetamidophenol reacted normally with (I*a*), prepared *in situ* from *N*-acetyl-3 : 5-dinitro-DL-tyrosine ethyl ester and toluene-*p*-sulphonyl chloride in pyridine, to yield the ether (II*a*). Catalytic reduction gave the diamine (III*a*), which could not be crystallised as such or in the form of various salts and acyl derivatives. Tetrazotisation of the crude diamine under anhydrous conditions, followed by treatment with sodium iodide, gave a high yield of the di-iododiphenyl ether (IV*a*), which was hydrolysed to the diamino-acid (V; X = H). Attempts to introduce iodine atoms *ortho* to the aromatic amino-group of this compound have all proved unavailing: iodine or iodine monochloride, under a variety of conditions, gave always either unchanged starting material or an intractable tar. Since the unprotected amino-acid grouping might be expected to be unstable in the more vigorous iodination conditions, several attempts were made to

* Part XII, *J.*, 1953, 764.

iodinate (IV*a*), in which all the functional groups are protected, but with no greater success.

It seemed desirable to attempt the iodination with a compound in which the aromatic amino-group was free, while the side-chain amino-acid group was protected in a stable derivative and to this end the hydantoin derivative (IV*b*) was prepared by reactions similar to those used in the preparation of (IV*a*). Hydrolysis with aqueous alcoholic hydrogen chloride gave the required amino-compound (VI), but here again treatment with iodine monochloride in acetic acid gave no crystalline product and the project was abandoned.

Of the methods considered for the preparation of the 4′-mercapto-analogue (XII; X = I) of thyroxine, those proceeding *via* (XII; X = H) were rejected, as it was considered that great difficulty would be encountered in the introduction of the 3′ : 5′-iodine atoms. Instead, routes to compounds of type (XII; X = NO_2) were investigated in the hope that the 3′ : 5′-nitro-groups would be replaceable by iodine atoms without undue complication.



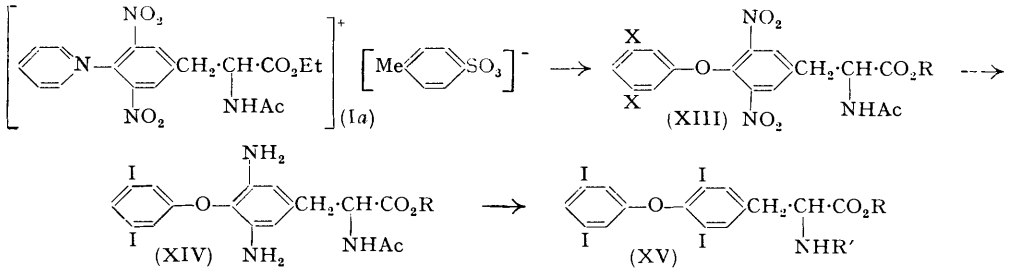
Chapman and Owen (*J.*, 1950, 579) have reported the preparation of alkyl thiolacetates by reaction of the corresponding alkyl toluene-*p*-sulphonate with potassium thioacetate. These authors showed that phenyl thiolacetate could not be prepared by this method, but it seemed probable that an aryl toluene-*p*-sulphonate suitably activated by nitro-groups might react as do the alkyl compounds. As an example 4-methyl-2 : 6-dinitrophenyl toluene-*p*-sulphonate was treated with potassium thioacetate under various conditions. With the thioacetate in excess in ethanol, the only product isolated was a compound of uncertain structure, but with one nitro-group reduced. When only one equivalent of potassium thioacetate was used, hydrolysis occurred and 4-hydroxy-3 : 5-dinitrotoluene was isolated. With acetone as solvent, however, and under carefully controlled conditions, the required 4-methyl-2 : 6-dinitrophenyl thiolacetate (VII) was obtained in fair yield.

In view of the ready reaction of 1-(2 : 6-dinitroaryl)pyridinium salts with phenols, the reaction of the quaternary salt (VIII) with thioacetic acid was investigated. When the latter was used in considerable excess, the product appeared to be 2 : 5-dimethyl-7-nitrobenzothiazole (IX), reaction of the required type having apparently occurred, followed by reduction of one of the nitro-groups and cyclisation. With only one equivalent of thioacetic acid and a short reaction time, some of the required compound (VII) could be isolated, but it was contaminated with the diphenyl sulphide (X), formed, presumably, by hydrolysis of (VII) and reaction with a further molecule of quaternary salt. With long reaction times the diphenyl sulphide was the only product isolated.

In order to apply these findings to the synthesis of (XII; X = NO_2), a suitable derivative of 3 : 5-di-iodo-3′ : 5′-dinitrothyronine was required. Nitration of *N*-acetyl-3 : 5-di-iodo-4-*p*-methoxyphenoxy-L-phenylalanine ethyl ester (XI; R = Me, X = H) (Part V, *J.*, 1949, 3424) in a mixture of sulphuric and acetic acids gave the 3′ : 5′-dinitro-derivative (XI; R = Me, X = NO_2). Alkyl aryl ethers containing two or more nitro-groups *ortho* or *para* to the ether linkage are known to be smoothly demethylated by pyridine (Kohn and Grauer, *Monatsh.*, 1913, **34**, 1751; Kohn and Loff, *ibid.*, 1924, **45**, 605; Cahn, *J.*, 1931, 630, 1121), and the required thyronine derivative (XI; R = H, X = NO_2) was obtained from its methyl ether in this way. Both the toluene-*p*-sulphonate, which could not be obtained crystalline, and the quaternary salt derived from it by treatment with pyridine were prepared, and these compounds were treated with potassium thio-

acetate and thioacetic acid respectively. Unfortunately, the conditions that led successfully to the thiophenol derivative in the model experiments gave only gums, from which no pure product could be isolated, and the investigation was carried no further.

Preparation of the " deoxythyroxine " (XV; R = R′ = H) was first attempted *via* the tetranitro-compound (XIII; R = Et, X = NO_2), which was prepared in the usual way from the quaternary salt (I*a*) and 3 : 5-dinitrophenol. The reaction was sluggish, and even after 66 hr. the yield was unusually low. In the presence of palladised charcoal, (XIII; R = Et, X = NO_2) absorbed hydrogen sufficient to reduce all four nitro-groups, but the product was very unstable and could not be crystallised or converted into a crystalline derivative. In spite of efforts to prevent aerial oxidation while the crude material was treated successively with nitrosylsulphuric acid and sodium iodide, none of the required tetra-iodo-compound (XV; R = Et, R′ = Ac) could be isolated from the tarry product.



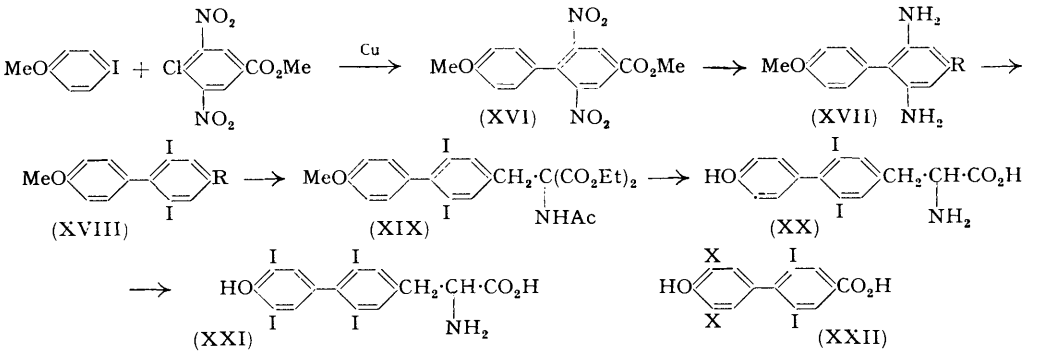
For an alternative method, involving stepwise introduction of the iodine atoms, 3 : 5-di-iodophenol was required, but neither of the methods of preparation described in the literature proved satisfactory (Willgerodt and Arnold, *Ber.*, 1901, **34**, 3343; Brenans, *Compt. rend.*, 1903, **136**, 236; *Bull. Soc. chim.*, 1903, **29**, 227; Hodgson and Wignall, *J.*, 1926, 2077). However, a simple and convenient method was found in the catalytic hydrogenation of 3 : 5-dinitroanisole, replacement of the resulting amino-groups by iodine, and demethylation with hydriodic acid.

Treatment of the quaternary salt (I*a*) with 3 : 5-di-iodophenol gave a satisfactory yield of *N*-acetyl-4-(3 : 5-di-iodophenoxy)-3 : 5-dinitro-DL-phenylalanine ethyl ester (XIII; X = I, R = Et). The nitro-groups in this compound could not be reduced catalytically without simultaneous removal of the iodine atoms, and several mild chemical reducing agents failed to affect the material or the corresponding acid. The required product was finally obtained by treatment of (XIII; X = I, R = Et) with iron powder in hydrochloric–acetic acids. Replacement of the amino-groups by iodine in the usual way, and hydrolysis, gave the amino-acid (XV; R = R′ = H).

For the preparation of the diphenyl derivative (XXI), a " deoxythyroxine " isomeric with (XV; R = R′ = H), *p*-iodoanisole and methyl 4-chloro-3 : 5-dinitrobenzoate were treated at 240° with copper bronze, to give methyl 4′-methoxy-2 : 6-dinitrodiphenyl-4-carboxylate (XVI) in *ca.* 50% yield. This result was not unexpected since, although the yields of unsymmetrical diaryls produced by the Ullmann method tend to be low owing to competing formation of the symmetrical compounds, several examples have been described of satisfactory reaction when one of the components has two bulky groups *ortho* to the halogen atom (Lesslie and Turner, *J.*, 1930, 1758; 1931, 1188; Rule and Smith, *J.*, 1937, 1096). A minor by-product of the reaction was methyl 3 : 5-dinitrobenzoate.

Catalytic hydrogenation of (XVI) gave the diamine (XVII; R = CO_2Me), which was converted *via* the tetrazonium salt, prepared under the usual anhydrous conditions, into the di-iodo-ester (XVIII; R = CO_2Me), from which (XXII; X = I) was prepared by standard methods. Although lithium aluminium hydride is known to be capable of reducing aromatic iodo-compounds (Trevoy and Brown, *J. Amer. Chem. Soc.*, 1949, **71**, 1675), it was hoped that selective reduction of the carbomethoxy-group of (XVIII; R = CO_2Me) might be possible under controlled conditions. Treatment of the ester with an

equimolecular quantity of lithium aluminium hydride at 0° gave a gum that, after acetylation, yielded crystalline (XVIII; R = CH_2·OAc) in only 33% of the theoretical amount. Reduction of the quantity of reducing agent left some ester unchanged, but had no effect on the net yield of the required material. Analysis of the crude products of such reductions showed that partial loss of iodine was occurring. Nevertheless, the crude alcohol (XVIII; R = CH_2·OH) obtained from a reduction was treated with thionyl chloride and the chloromethyl compound (XVIII; R = CH_2Cl), again without purification, was condensed with sodio-acetamidomalonic ester to give a rather poor yield of (XIX). The malonic ester (XIX) was stripped of its protecting groups by means of a mixture of hydriodic and acetic acids. Iodination of the resulting amino-acid (XX) gave the required 4-(4-hydroxy-3 : 5-di-iodophenyl)-3 : 5-di-iodo-DL-phenylalanine (XXI).



Because of the unsatisfactory reduction stage in the synthesis described above, some experiments were carried out on an alternative route which avoided the necessity for selective reduction. The di-amino-ester (XVII; R = CO_2Me) was smoothly reduced with excess of lithium aluminium hydride to the alcohol (XVII; R = CH_2·OH). Tetrazotisation of this compound with nitrosylsulphuric acid in acetic acid followed by decomposition with iodide gave a fair yield of (XVIII; R = CH_2·OAc), acetylation of the hydroxyl group having occurred during tetrazotisation. Alkaline hydrolysis of this acetate gave the pure alcohol (XVIII; R = CH_2·OH).

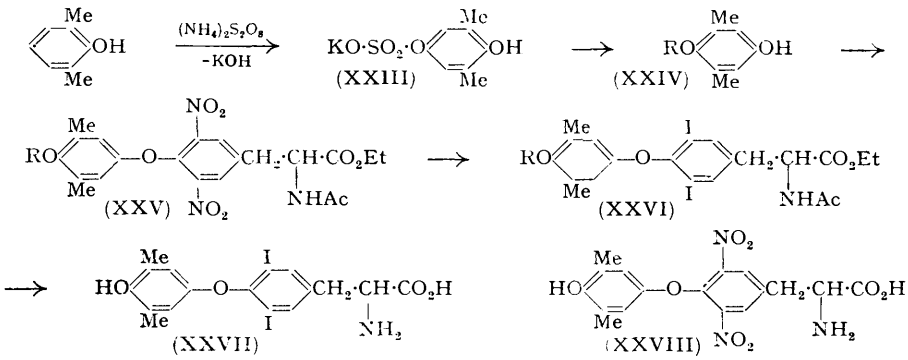
Substitution of the methyl groups of riboflavin by chlorine atoms leads to a compound that antagonises the action of the vitamin (Kuhn, Weygand, and Möller, *Ber.*, 1943, **76**, 1044). This may well be due to the similarity in the dimensions of the methyl group and the chlorine atom, but we have nevertheless investigated the preparation of compounds in which some or all of the iodine atoms of thyroxine are replaced by methyl groups, in the hope that an antagonist might result.

2 : 6-Dimethylquinol was prepared from 2 : 6-dimethylphenol by oxidation with ammonium persulphate and hydrolysis of the sulphate (XXIII) so formed (Baker and Brown, *J.*, 1948, 2303). Contrary to the experience of these authors we find ammonium persulphate to be preferable to the potassium salt, which gave rather lower yields and was inconveniently insoluble.

Reaction of 2 : 6-dimethylquinol with the quaternary salt (I*a*) gave a mixture that, after chromatography and repeated recrystallisation, yielded a small quantity of the required diphenyl ether (XXV; R = H). It seemed probable that this compound, formed by reaction of the unhindered hydroxyl group of 2 : 6-dimethylquinol, would be obtained in preference to the isomeric *N*-acetyl-4-(4-hydroxy-2 : 6-dimethylphenoxy)-3 : 5-dinitro-DL-phenylalanine ethyl ester, and evidence that the compound isolated was in fact (XXV; R = H) was provided by comparison of the free amino-acid (XXVIII), obtained from it by hydrolysis, with an authentic specimen prepared by the method described below. The amino-acid melted with decomposition, and the mixed melting point test was not, therefore, wholly satisfactory, but further evidence was provided by the fact that the compound gave no colour when treated with benzene- or *p*-nitrobenzene-diazonium chloride, indicating the absence of an unsubstituted position

ortho to the phenolic hydroxyl. The presence of the isomeric compound among the products of the above reaction is certainly not excluded, and its presence may, indeed, account for the difficulty encountered in isolating (XXV; R = H) in a pure state.

In view of the unsatisfactory nature of the above reaction, the 1-hydroxy-group of 2 : 6-dimethylquinol was then blocked before reaction with the quaternary salt. Attempts to acetylate or benzoylate the free hydroxy-group of (XXIII) led, after removal of the sulphate ester grouping, only to some 2 : 6-dimethylquinol. Treatment with methyl sulphate (Baker and Brown, *loc. cit.*) and acid hydrolysis gave a moderate yield of 4-methoxy-3 : 5-dimethylphenol (XXIV; R = Me); benzyl chloride gave the corresponding benzyl ether (XXIV; R = CH_2Ph), but in only 18% yield. Comparison of the last result with the quantitative yield obtained by benzylation of the isomeric potassium 4-hydroxy-2 : 6-dimethylphenyl sulphate (Baker and Brown, *loc. cit.*) shows the effect of the two *o*-methyl groups.



The quaternary salt (I*a*) reacted with (XXIV; R = Me) to give the diphenyl ether (XXV; R = Me) and with (XXIV; R = CH_2Ph) to give (XXV; R = CH_2Ph). Earlier experiments (Part VIII, *J.*, 1951, 2467) have shown that 4′-methoxy-2 : 6-dinitrodiphenyl ethers are not readily demethylated, but it was found that the more labile benzyloxy-group of (XXV; R = CH_2Ph) could be cleaved by boiling aqueous alcoholic hydrochloric acid, to give 3′ : 5′-dimethyl-3 : 5-dinitro-DL-thyronine (XXVIII).

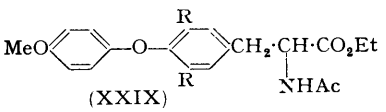
The methoxy-compound (XXV; R = Me) was reduced catalytically, and the diamine, without isolation, was converted into the di-iodo-compound (XXVI) in the usual way. Treatment of this compound with hydriodic acid gave the required 3 : 5-di-iodo-3′ : 5′-dimethyl-DL-thyronine (XXVII), though here again the effect of the methyl groups was shown by the sluggishness of the reaction.*

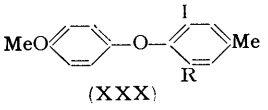
The 3 : 5-dimethyl analogue of thyroxine proved, as expected, to be much less accessible than the isomer already described. However, although the required compound was not synthesised, we record here the results of preliminary experiments for the light they throw on the preparation of 2 : 6-dimethyldiphenyl ethers.

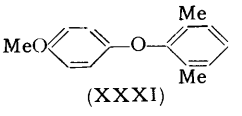
The ease with which 2 : 6-di-iododiphenyl ethers can be prepared made these appear attractive intermediates for the preparation of the dimethyl compounds. The first method examined for the replacement of iodine atoms by methyl groups proceeded *via* the dinitrile. *N*-Acetyl-3 : 5-dicyano-4-*p*-methoxyphenoxy-L-phenylalanine ethyl ester (XXIX; R = CN) was readily prepared by treatment of the 3 : 5-di-iodo-compound (XXIX; R = I) (Part V, *J.*, 1949, 3424) with cuprous cyanide in boiling pyridine. An attempt to prepare the same compound from (XXIX; R = NH_2) (Part V, *loc. cit.*) by tetrazotisation, followed by treatment with sodium cuprocyanide, was unsuccessful. It was hoped that the cyano-groups could be reduced to aminomethyl groups, which should then be convertible into methyl groups, but several attempts to reduce (XXIX; R = CN) catalytically failed to produce a pure product. In view of the very smooth reaction of (XXIX; R = I) with cuprous cyanide, it is surprising that several rather similar

* Since this manuscript was prepared, Bruice, Kharasch, and Winzler (*J. Org. Chem.*, 1953, **18**, 83) have described the preparation of the L-isomer of (XXVII) by a similar method.

compounds, namely, L-thyroxine, *N*-acetyl-L-thyroxine, *ON*-diacetyl-3 : 5-di-iodo-L-tyrosine ethyl ester, and *N*-acetyl-3 : 5-di-iodo-*O*-methyl-L-tyrosine ethyl ester, either failed to react under similar conditions or yielded products from which no pure material could be isolated.


(XXIX)

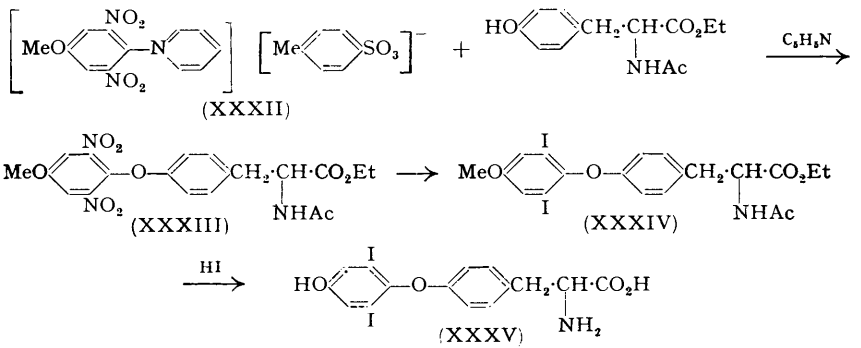

(XXX)


(XXXI)

Another method considered for converting 2 : 6-di-iododiphenyl ethers into the dimethyl compounds involved methylation of the Grignard compound derived from the former. However, carboxylation of 3 : 5-di-iodo-4'-methoxy-4-methyldiphenyl ether (XXX; R = I) after treatment with magnesium and some methyl iodide gave an iodo-acid (XXX; R = CO_2H), indicating that only one iodine atom had reacted.

It was expected that the conditions required for reaction of a 2 : 6-dimethylphenol with an unactivated aromatic halide would be so vigorous that, if a thyroxine analogue were to be prepared, the alanine side-chain would have to be built up subsequently from a simple, stable group such as carbethoxy. In accordance with this, 2 : 6-dimethylphenol, itself, could not be made to react with bromobenzene in the Ullmann reaction, but the more reactive halogen of *p*-iodoanisole permitted reaction, and a moderate yield of 4'-methoxy-2 : 6-dimethyldiphenyl ether (XXXI) resulted after prolonged treatment with 2 : 6-dimethylphenol in the presence of potassium carbonate and copper bronze at 220—240°.* This experiment demonstrates that such an approach to the 3 : 5-dimethyl analogue of thyroxine is feasible, but we were unable to carry the investigation beyond this point.

Finally, we describe the preparation of the known 2' : 6'-di-iodothyronine (XXXV) by a new route. The DL-form of this amino-acid was described by Niemann and McCasland (*J. Amer. Chem. Soc.*, 1944, **66**, 1870). The essential stages of their synthesis were the reaction of 3 : 4 : 5-tri-iodo-nitrobenzene with *N*-benzoyl-DL-tyrosine ethyl ester and replacement of the 4'-nitro-group in the resulting diphenyl ether by a hydroxy-group, *via* the amino- and diazonium compounds. Cortell (*J. Clin. Endocrinol.*, 1949, **9**, 955) reported that (XXXV) antagonises the effect of thyroxine in thiouracil-treated rats, as shown by the thyroid weight. We have now prepared both the L- and the DL-form of the compound in order to investigate their activity in the oxygen-consumption test. The


(XXXII)

(XXXIII)

(XXXIV)

(XXXV)

synthesis, an adaptation of our normal method for preparing di-iododiphenyl ethers, is illustrated by the formulæ (XXXII)—(XXXV). *N*-Acetyl-L-tyrosine ethyl ester was used as starting material, and the DL-acid (XXXV) was prepared by racemising the acetamido-ester (XXXIV) with sodium methoxide and treating the product with hydriodic acid. The melting point of our material was substantially higher than that reported by Niemann and McCasland (*loc. cit.*).

* Bruice, Kharasch, and Winzler (*loc. cit.*) have described the preparation of 4'-methoxy-2 : 6 : 3' : 5'-tetramethyldiphenyl ether by heating a mixture of 4-bromo-2 : 6-dimethylanisole, 2 : 6-dimethylphenol, potassium hydroxide, and copper bronze at 235° for 3 hr.

Biological Results.—The various thyroxine analogues and certain of the intermediates reported in this paper have been examined for anti-thyroid activity by the method outlined in Part XII. All were inactive with the exception of compound (XXII; X = H) and DL-2′ : 6′-di-iodothyronine (XXXV), both of which showed slight activity. The tests were partly done in the pharmacological laboratories of the Research Division of Glaxo Laboratories Ltd., where they were under the direction of Mr. E. G. Tomich, and partly by Professor N. F. Maclagan and his colleagues at Westminster Medical School, to whom we are indebted for their assistance.

Experimental

4-p-*Acetamidophenoxy*-N-*acetyl*-3 : 5-*dinitro*-DL-*phenylalanine Ethyl Ester* (II*a*).—*N*-Acetyl-3 : 5-dinitro-DL-tyrosine ethyl ester (Part VIII, *J.*, 1951, 2467) (16 g.) and toluene-*p*-sulphonyl chloride (9·8 g.) in pyridine (70 c.c.) were kept at 110° for 1 hr. *p*-Acetamidophenol (21 g.) was added and the mixture boiled gently under reflux for 2 hr. The cooled solution was poured into a large volume of dilute hydrochloric acid, from which the product was extracted with two portions of ethyl acetate. After being washed with very dilute sodium hydroxide solution and water the solution was evaporated to dryness. The residual oil crystallised when seeded. However, recrystallisation was difficult and did not give a sharply melting product. The material was, therefore, redissolved in dry ethyl acetate and passed down a column of activated alumina. Recrystallisation of the most easily eluted fractions from alcohol gave the *diphenyl ether* (13 g., 58%), m. p. 175—177°. Repeated recrystallisation from alcohol or aqueous methanol yielded pale yellow needles, m. p. 178° (Found: C, 53·2; H, 4·8. $C_{21}H_{22}O_9N_4$ requires C, 53·15; H, 4·7%).

When this diacetamido-ester was boiled in aqueous-alcoholic hydrochloric acid for 2 hr., the *diamino-acid dihydrochloride* separated on cooling. After crystallisation from fairly concentrated hydrochloric acid it formed almost colourless needles, beginning to blacken at about 250° (Found: C, 41·2; H, 3·8; N, 12·75. $C_{15}H_{14}O_7N_4$,2HCl requires C, 41·4; H, 3·7; N, 12·9%).

4-p-*Acetamidophenoxy*-N-*acetyl*-3 : 5-*di-iodo*-DL-*phenylalanine Ethyl Ester* (IV*a*).—The dinitro-compound (8·1 g.) in alcohol (220 c.c.) rapidly absorbed hydrogen when stirred under hydrogen at 80 atm. in the presence of palladised charcoal (10%; 1 g.). Filtration through kieselguhr and distillation of the alcohol at reduced pressure in an atmosphere of carbon dioxide left the 3 : 5-diamine as an almost colourless gum.

The gum, dissolved in acetic acid (23 c.c.), was slowly added to sulphuric acid (12 c.c.) cooled below −5°. This solution was added during 45 min. to a solution of sodium nitrite (2·8 g.) in a mixture of sulphuric acid (30 c.c.) and acetic acid (50 c.c.), well stirred at <0°. After another hr. at 0° the mixture was added during 15 min. to a stirred solution of sodium iodide (18 g.), iodine (15 g.) and urea (1 g.) in water (300 c.c.) and chloroform (100 c.c.) at 35°. After an hr. the chloroform was separated and the lower, black, viscous layer and the upper, aqueous layer were extracted with more chloroform. However, evaporation of the chloroform left only a small amount of material. The lower layer was, therefore, shaken with sodium iodide solution, sodium metabisulphite solution, and then water. The last of the iodine was removed by dissolving the material in alcohol and treating the solution with sulphur dioxide and sodium hydrogen carbonate. Pouring the solution into water gave a sticky gum which crystallised when warmed and seeded. The product was ground under water, washed, and dried; it then had m. p. 211—216°. Recrystallisation from alcohol and twice from aqueous dioxan gave the *di-iodo*-compound (5·0 g., 46%) as colourless needles, m. p. 224—225° (Found: C, 39·5; H, 3·5. $C_{21}H_{22}O_5N_2I_2$ requires C, 39·6; H, 3·5%).

4-p-*Aminophenoxy*-3 : 5-*di-iodo*-DL-*phenylalanine* (V; X = H).—The above diacetamido-ester (1 g.), alcohol (6 c.c.), water (3·5 c.c.), and concentrated hydrochloric acid (3·5 c.c.) were boiled for 3 hr. Concentrated hydrochloric acid (1 c.c.) was added and the needles of the *diamino-acid dihydrochloride* (0·8 g.; 85%) which had separated were filtered off and washed with dilute hydrochloric acid. Recrystallisation from 2N-hydrochloric acid yielded fine colourless needles, beginning to decompose at 250° (Found: C, 30·3; H, 3·0. $C_{15}H_{14}O_3N_2I_2$,2HCl requires C, 30·15; H, 2·7%).

DL-5-(4-p-*Acetamidophenoxy*-3 : 5-*dinitrobenzyl*)*hydantoin* (II*b*).—DL-5-(4-Hydroxy-3 : 5-dinitrobenzyl)hydantoin (Part III, *J.*, 1949, S 199) (10 g.) and toluene-*p*-sulphonyl chloride (6·5 g., 1 mol.) in pyridine (40 c.c.) were heated at 100° for 45 min. After the addition of *p*-acetamidophenol (15 g., 3 mol.) to the cooled solution the mixture was reheated for 2 hr.,

cooled, and poured into dilute hydrochloric acid. The solid was filtered off, washed, dissolved in the minimum amount of dilute sodium hydroxide solution, and, after filtration of the solution, reprecipitated with dilute hydrochloric acid. Recrystallisation from acetic acid (charcoal) produced the pure *ether* (8·6 g., 60%) as a yellow powder, melting with effervescence at 275° (Found: C, 50·4; H, 3·6; N, 16·4. $C_{18}H_{15}O_8N_5$ requires C, 50·35; H, 3·5; N, 16·3%).

DL-5-(4-p-*Acetamidophenoxy*-3 : 5-*di-iodobenzyl*)*hydantoin* (IV*b*).—The foregoing dinitro-compound (1 g.) was hydrogenated in acetic acid (50 c.c.) at ordinary temperature and pressure in the presence of palladised charcoal (0·5 g.). The theoretical amount of hydrogen was taken up in 6 hr. The solution, after removal of the catalyst, was added to sulphuric acid (30 c.c.) kept at about 10°. A solution of sodium nitrite (0·4 g.) in sulphuric acid (50 c.c.) and acetic acid (50 c.c.) was cooled to −5° and stirred whilst the diamine solution was added dropwise during 30 min. The mixture was then stirred at 0° for 1½ hr. more and added dropwise during 10 min. to a stirred solution of iodine (5 g.) and sodium iodide (5 g.) in water (200 c.c.). The mixture was kept for 2 hr. and the black solid was filtered off and treated with sodium meta-bisulphite solution to remove free iodine, a buff-coloured solid resulting. After drying, this was chromatographed in acetone (100 c.c.) on alumina. A mixture of acetone (90%), methanol (5%), and acetic acid (5%) eluted a solid which after crystallisation from a little acetic acid gave the *di-iodo*-compound (0·86 g., 57%) as pale buff-coloured crystals, m. p. 290° after sintering at 160° (Found: N, 6·5; I, 38·8. $C_{18}H_{15}O_4N_3I_2,CH_3{\cdot}CO_2H$ requires N, 6·5; I, 39·0%).

DL-5-(4-p-*Aminophenoxy*-3 : 5-*di-iodobenzyl*)*hydantoin* (VI).—A solution of the *N*-acetyl derivative (3·3 g.) in ethanol (50 c.c.) and concentrated hydrochloric acid (10 c.c.) was boiled under reflux for 45 min. After cooling, the solid was filtered off and dissolved in hot water (100 c.c.). The solution was decolorised with charcoal, filtered, and brought to pH 5 by addition of sodium acetate solution. The *amine* was precipitated as a pale buff-coloured powder, m. p. 261—264°. Crystallisation from aqueous ethanol gave almost colourless crystals (1·7 g., 59%), m. p. 210° (Found: C, 35·2; H, 3·0; N, 7·3; I, 44·3. $C_{16}H_{13}O_3N_3I_2,\frac{1}{2}EtOH$ requires C, 35·7; H, 2·8; N, 7·3; I, 44·4%).

4-*Methyl*-2 : 6-*dinitrophenyl Thiolacetate* (VII).—3 : 5-Dinitro-*p*-tolyl toluene-*p*-sulphonate (1 g.) was dissolved in boiling acetone (25 c.c.), potassium thioacetate (0·32 g.) was then added, and refluxing continued for a few min., until there was no further separation of solid. On dilution of the cooled mixture with water yellow crystals slowly separated. After an hour this material was collected and crystallised from light petroleum (b. p. 80—100°). The *thiolacetate* (350 mg., 48%) separated as pale yellow plates, m. p. 132—134° (Found: C, 42·2; H, 3·2; N, 11·0; S, 12·3. $C_9H_8O_5N_2S$ requires C, 42·2; H, 3·2; N, 11·0; S, 12·5%).

Reaction between N-(4-*Methyl*-2 : 6-*dinitrophenyl*)*pyridinium Toluene*-p-*sulphonate and Excess of Thioacetic Acid.*—The pyridinium salt (Part II, *J.*, 1949, S 190) (1·05 g.), thioacetic acid (0·56 c.c., 3 mols.), dry chloroform (10 c.c.), and dry pyridine (3 c.c.) were refluxed together for 12 hr. Evaporation to dryness under diminished pressure left an orange-red oil which solidified. This material was dissolved in ethanol (10 c.c.) and filtered from sulphur, and the clear solution diluted with water (50 c.c.). The solid was collected, dried, and crystallised from aqueous ethanol from which 2 : 5-*dimethyl*-7-*nitrobenzothiazole* (0·22 g.) separated as faintly yellow needles, m. p. 136—138° (Found: C, 52·1; H, 4·0; S, 15·2. $C_9H_8O_2N_2S$ requires C, 51·9; H, 3·9; S, 15·4%).

The material was insoluble in dilute hydrochloric acid, but dissolved in the concentrated acid from which it was precipitated unchanged by dilution with water.

Reaction between N-(4-*Methyl*-2 : 6-*dinitrophenyl*)*pyridinium Toluene*-p-*sulphonate and One Molecular Proportion of Thioacetic Acid.*—(*a*) A mixture of the pyridinium salt (1·25 g.), thio-acetic acid (0·21 c.c., 1 mol.), dry chloroform (10 c.c.), and dry pyridine (3 c.c.) was refluxed for 2 hr. A yellow solid separated during this time. The mixture was evaporated to smaller bulk, diluted with water, and extracted with chloroform (3 × 40 c.c.). Evaporation left a yellow solid (0·13 g., 11%) which was washed with a little ethanol and dried (m. p. 272—275°). Crystallisation from chloroform–light petroleum (b. p. 60—80°) gave *di*-(4-*methyl*-2 : 6-*dinitro-phenyl*) *sulphide* (X) as a yellow solid, m. p. 274—275° (Found: C, 43·0; H, 2·9; N, 14·4; S, 8·2. $C_{14}H_{10}O_8N_4S$ requires C, 42·6; H, 2·6; N, 14·2; S, 8·1%).

When the reaction was carried out in dry pyridine for 48 hr. at room temperature, a similar yield of the same product was obtained.

(*b*) The pyridinium salt (1·1 g.) and thioacetic acid (0·185 c.c., 1 mol.) in dry chloroform (25 c.c.) were heated to boiling, and then dry pyridine (1 c.c.) was added. All the solid rapidly dissolved and the solution was then evaporated to dryness under reduced pressure. The

residual oil solidified on trituration with water. The dried solid was extracted with light petroleum (b. p. 80—100°), and the extract, on cooling, gave 4-methyl-2 : 6-dinitrophenyl thiolacetate (120 mg.), m. p. 121—124°. Further crystallisation raised the m. p. to 132—134°.

The solid left from the extraction crystallised from chloroform–light petroleum, giving di-(4-methyl-2 : 6-dinitrophenyl) sulphide (130 mg.), m. p. 264—266°.

N-*Acetyl*-3 : 5-*di-iodo*-4-(4-*methoxy*-3 : 5-*dinitrophenoxy*)-L-*phenylalanine Ethyl Ester* (XI; R = Me, X = NO_2).—A suspension of *N*-acetyl-3 : 5-di-iodo-4-*p*-methoxyphenoxy-L-phenylalanine ethyl ester (Part V, *J.*, 1949, 3424) (5 g.) in glacial acetic acid (10 c.c.) was treated slowly below 25° with concentrated sulphuric acid (20 c.c.). The resulting clear solution was stirred and kept between 5° and 10° while nitric acid (*d* 1·42; 1·2 c.c.) was added dropwise. When addition was complete, the mixture was stirred for a further 20 min. and then poured on crushed ice. The solid was washed with water, dried, and crystallised from aqueous ethanol from which the *dinitro*-compound (3·8 g., 66%) separated as aggregates of soft needles, m. p. 167—168°, $[\alpha]_D^{22}$ +19·5° (*c*, 1·98 in dioxan) (Found: C, 34·5; H, 2·7; N, 6·1; I, 36·5. $C_{20}H_{19}O_9N_3I_2$ requires C, 34·4; H, 2·7; N, 6·0; I, 36·3%).

N-*Acetyl*-3 : 5-*di-iodo*-3′ : 5′-*dinitro*-L-*thyronine Ethyl Ester* (XI; R = H, X = NO_2).—A solution of the foregoing ether (3·7 g.) in pyridine (40 c.c.) was refluxed for 1 hr., during which a deep red colour developed. After cooling, dilution with dry ether precipitated a red solid which appeared to be deliquescent. The liquid phase was decanted and the residue washed twice with dry ether. 2N-Sulphuric acid (100 c.c.) was then added and the mixture warmed gently to remove traces of ether. The solid was ground under the acid until all the red product had disappeared. The bright yellow material remaining was collected, washed with water, dried, and crystallised from aqueous dioxan, from which N-*acetyl*-3 : 5-*di-iodo*-3′ : 5′-*dinitro*-L-*thyronine ethyl ester* (2·1 g., 51%) separated as yellow needles. The compound, which contained a molecule of dioxan of crystallisation, melted first at 115°, resolidified, and remelted at 161—163°, $[\alpha]_D^{19}$ +17·6° (*c*, 1·06 in dioxan) (Found: C, 35·5; H, 3·3; N, 5·4; I, 32·9. $C_{19}H_{17}O_9N_{23}I,C_4H_8O_2$ requires C, 35·8; H, 3·3; N, 5·4; I, 32·8%).

3 : 5-*Di-iodo*-3′ : 5′-*dinitro*-L-*thyronine*.—The above acetamido-ester (1 g.), concentrated hydrochloric acid (5 c.c.), and acetic acid (5 c.c.) were refluxed for 2 hr. On cooling, a yellow solid separated and this was collected and dried. It was purified by dissolving it in acetic acid containing a little hydrochloric acid, and carefully adding water. The *amino-acid* (0·6 g., 75%) separated as yellow needles, m. p. 270° (decomp.) (Found: C, 29·6; H, 2·0; I, 42·0. $C_{15}H_{11}O_8N_3I_2$ requires C, 29·3; H, 1·8; I, 41·3%).

N-*Acetyl*-4-(3 : 5-*dinitro*-4-*toluene*-p-*sulphonyloxyphenoxy*)-3 : 5-*di-iodo*-L-*phenylalanine Ethyl Ester* (XI; R = *p*-Me·C_6H_4·SO_2, X = NO_2).—*N*-Acetyl-3 : 5-di-iodo-3′ : 5′-dinitro-L-thyronine ethyl ester (1·0 g.) and toluene-*p*-sulphonyl chloride (0·28 g.) were dissolved in acetone (10 c.c.), and N-sodium hydroxide (1·46 c.c.) was added slowly with stirring. After 2 hr., the bulk of the acetone was evaporated off under diminished pressure and the residue diluted with water. The oil first formed solidified when scratched, and this solid was ground with water, collected, and dried. The toluene-*p*-sulphonate was a pale yellow solid, m. p. *ca.* 85°, which could not be crystallised.

N-*Acetyl*-4-(3 : 5-*dinitrophenoxy*)-3 : 5-*dinitro*-DL-*phenylalanine Ethyl Ester* (XIII; X = NO_2, R = Et).—A solution of *N*-acetyl-3 : 5-dinitro-DL-tyrosine ethyl ester (10 g.) and toluene-*p*-sulphonyl chloride (6·25 g.) in acetone (60 c.c.) was stirred while 2N-sodium hydroxide (15 c.c.) was added dropwise during 1 hr. After a further 2 hr. stirring the mixture was evaporated nearly to dryness and the residue dissolved in benzene (125 c.c.). The solution was washed successively with water, N-sodium hydroxide, and water, dried, and evaporated to about 10 c.c. Pyridine (6·8 c.c.) was then added and the solution refluxed for 30 min. during which the quaternary salt separated as a sticky gum, from which the benzene was decanted. The gum was dissolved in a mixture of chloroform (50 c.c.) and pyridine (15 c.c.) and, after the addition of 3 : 5-dinitrophenol (17 g.), the solution refluxed for 66 hr. After dilution with chloroform (500 c.c.) the solution was washed successively with N-hydrochloric acid, water, N-sodium hydroxide, and water. Evaporation of the dried solution left a white solid which crystallised from ethanol, giving the *tetranitro*-compound (6·8 g., 45%) as needles, m. p. 183—184° (Found: C, 45·2; H, 3·6; N, 13·5. $C_{19}H_{17}O_{12}N_5$ requires C, 45·0; H, 3·4; N, 13·8%).

3 : 5-*Di-iodoanisole*.—3 : 5-Dinitroanisole (*Org. Synth.*, Coll. Vol. I, 2nd Edn., p. 219) (16 g.) in glacial acetic acid (300 c.c.) was hydrogenated at ordinary temperature and pressure in the presence of Adams's catalyst. Subsequent operations were conducted in an atmosphere of carbon dioxide. After filtration, the yellow filtrate was evaporated under reduced pressure.

The residual sticky brown oil was dissolved in glacial acetic acid (40 c.c.) and cautiously added to concentrated sulphuric acid (40 c.c.), excessive rise in temperature being avoided. This mixture was added dropwise during 30 min. at 5° to a stirred solution prepared by dissolution of sodium nitrite (13·2 g.) in concentrated sulphuric acid (40 c.c.; pre-cooled to 0°) and dilution with glacial acetic acid (80 c.c.). After a further hr. stirring the solution was added to a vigorously stirred mixture of sodium iodide (60 g.), iodine (50 g.), urea (40 g.), water (500 c.c.), and chloroform (250 c.c.); the temperature rose to *ca.* 35°. Stirring was continued for 1 hr., then the chloroform layer was collected and the aqueous residue extracted with more chloroform (3 × 100 c.c.). The extract was washed with water, covered with a solution of sodium metabisulphite, and sulphur dioxide was bubbled through it until the excess of iodine was removed. The solution was then washed with water, dried, and evaporated, to leave a yellow solid. Crystallisation from ethanol gave 3 : 5-di-iodoanisole (13·9 g., 48%) as tablets, m. p. 83—84° (Found : I, 70·3. Calc. for $C_7H_6OI_2$: I, 70·5%). (Hodgson and Wignall, *J.*, 1926, 2077, give m. p. 85°.)

3 : 5-*Di-iodophenol.*—3 : 5-Di-iodoanisole (10 g.) in 57% hydriodic acid (30 c.c.) and glacial acetic acid (45 c.c.) was refluxed for 2 hours. The solution was poured into water, and the solid extracted into chloroform. The oily solid left after removal of the solvent was crystallised from light petroleum (b. p. 80—100°), to give 3 : 5-di-iodophenol (7·2 g., 75%) as needles, m. p. 102—104° (Brenans, *Compt. rend.*, 1903, **136**, 236, gives m. p. 103—104°).

N-*Acetyl*-4-(3 : 5-*di-iodophenoxy*)-3 : 5-*dinitro*-DL-*phenylalanine Ethyl Ester* (XIII; X = I, R = Et).—The quaternary salt (I*a*) was prepared as described above from *N*-acetyl-3 : 5-dinitro-DL-tyrosine ethyl ester (3 g.). The gum was dissolved in dry chloroform (30 c.c.) and pyridine (12 c.c.), and 3 : 5-di-iodophenol (9 g.) was added. After 48 hr. refluxing, the product was isolated as described for compound (XIII; X = NO_2, R = Et). The *diphenyl ether* (4 g., 68%) separated from ethanol as needles, m. p. 201° (Found : C, 34·3; H, 2·7; N, 6·2. $C_{19}H_{17}O_8N_3I_2$ requires C, 34·1; H, 2·6; N, 6·3%).

From the alkaline washings most of the unused 3 : 5-di-iodophenol could be recovered by acidification.

4-(3 : 5-*Di-iodophenoxy*)-3 : 5-*dinitro*-DL-*phenylalanine.*—The above acetamido-ester (2 g.), glacial acetic acid (20 c.c.), and concentrated hydrochloric acid (20 c.c.) were refluxed for 2 hr. then diluted with a little water. The solid which had separated was collected and dried. Crystallisation from aqueous pyridine gave the *amino-acid* (1·7 g., 95%) as plates, m. p. 227° (decomp.) (Found : C, 30·0; H, 2·2; N, 6·6; I, 41·8. $C_{15}H_{11}O_7N_3I_2$ requires C, 30·2; H, 1·9; N, 7·0; I, 42·3%).

N-*Acetyl*-4-(3 : 5-*di-iodophenoxy*)-3 : 5-*dinitro*-DL-*phenylalanine* (XIII; X = I, R = H).—A solution of the foregoing amino-acid (0·5 g.) in N-sodium hydroxide (3·5 c.c.) was treated with acetic anhydride (0·25 c.c.), in portions, with vigorous shaking. A solid separated after a few min. and, after gentle warming (*ca.* 40°) to destroy excess anhydride, the mixture was acidified with hydrochloric acid, and the solid collected, washed with water, and dried. Crystallisation from glacial acetic acid gave the *acetamido-acid* as pale yellow needles, m. p. 245—246° (Found : C, 32·0; H, 2·1; N, 6·3; I, 39·0. $C_{17}H_{13}O_8N_3I_2$ requires C, 31·8; H, 2·0; N, 6·6; I, 39·6%).

N-*Acetyl*-4-(3 : 5-*di-iodophenoxy*)-3 : 5-*di-iodo*-DL-*phenylalanine Ethyl Ester* (XV; R = Et, R′ = Ac).—*N*-Acetyl-4-(3 : 5-di-iodophenoxy)-3 : 5-dinitro-DL-phenylalanine ethyl ester (5 g.) was dissolved in boiling glacial acetic acid (300 c.c.) and then stirred while the temperature dropped to 30°. All subsequent operations were carried out in an atmosphere of carbon dioxide. Iron powder (10 g.) was added in one portion, followed by concentrated hydrochloric acid (60 c.c.) in 5-c.c. portions during an hr., too great a rise in temperature being avoided. The mixture was stirred for a further hr., filtered to remove inorganic residues, and concentrated to small bulk (20 c.c.) under reduced pressure. An excess of saturated sodium hydrogen carbonate solution was added, then, after being shaken with chloroform (200 c.c.), the whole mixture was filtered to remove some dark insoluble material. The chloroform extract was dried and evaporated, leaving the diamine as a light brown glass, m. p. *ca.* 95°. The product was dissolved in a mixture of glacial acetic acid (25 c.c.) and concentrated sulphuric acid (25 c.c.) and added dropwise during 30 min. to a cooled, stirred solution of sodium nitrite (1·0 g.) in concentrated sulphuric acid (15 c.c.) diluted with acetic acid (15 c.c.). After being stirred for a further 2 hr. at 0°, the solution was added to sodium iodide (3 g.), iodine (3·5 g.), urea (5 g.), water (150 c.c.), and chloroform (200 c.c.) with vigorous stirring. The temperature rose to *ca.* 40° and after an hr. the chloroform layer was run off and the aqueous residue extracted with more chloroform. Excess of iodine was removed by treatment with sodium

metabisulphite solution. The combined chloroform layers gave, on evaporation, a yellow brown solid which was dissolved in boiling ethanol. The *tetra-iodo*-compound (2·44 g., 39%) separated as needles, m. p. 184—186° (Found: C, 27·7; H, 2·3; N, 1·7; I, 60·6. $C_{19}H_{17}O_4NI_4$ requires C, 27·5; H, 2·1; N, 1·7; I, 61·1%).

4-(3 : 5-*Di-iodophenoxy*)-3 : 5-*di-iodo*-DL-*phenylalanine* (XV; R = R′ = H).—A solution of the above acetamido-ester (1 g.) in a mixture of acetic acid (10 c.c.) and concentrated hydrochloric acid (10 c.c.) was boiled under reflux for 2 hr. A little water was added and the solid was filtered off and dried. It was purified by dissolving it in a boiling mixture of equal volumes of ethanol and 2N-sodium hydroxide and adding 2N-hydrochloric acid until the pH was 4—5. The *amino-acid* (0·61 g., 67%) melted at 223—225° (decomp.) (Found: C, 24·1; H, 1·9; N, 1·8; I, 65·6. $C_{15}H_{11}O_3NI_4$ requires C, 23·7; H, 1·5; N, 1·8; I, 66·7%).

Methyl 4′-*Methoxy*-2 : 6-*dinitrodiphenyl*-4-*carboxylate* (XVI).—A mixture of methyl 4-chloro-3 : 5-dinitrobenzoate (65 g.) and *p*-iodo-anisole (75 g.) was heated at 230—240° and copper-bronze (75 g.) was added in portions. The mixture was then kept at 235—240° for 30 min. After cooling, the solid was extracted with boiling acetone, and the extract was evaporated to dryness. The residual brown gum was dissolved in benzene and chromatographed on alumina. The solid residue obtained by evaporation of the benzene eluate was crystallised from alcohol, to give 40·9 g. (49%) of *methyl* 4′-*methoxy*-2 : 6-*dinitrodiphenyl*-4-*carboxylate*, m. p. 132—133°. Further crystallisation from alcohol gave yellow needles, m. p. 133—135° (Found: C, 54·3; H, 3·5; N, 8·4. $C_{15}H_{12}O_7N_2$ requires C, 54·2; H, 3·6; N, 8·4%).

The mother-liquors from the crystallisation of the crude diphenyl gave a small quantity of methyl 3 : 5-dinitrobenzoate, m. p. 110—112°, alone or mixed with an authentic specimen.

The diphenyl derivative (0·5 g.) was hydrolysed under reflux with concentrated hydrochloric acid (2·5 c.c.) and acetic acid (2·5 c.c.). The ester dissolved during about 1 hr. and after another 30 min. the solution was diluted with water to give an oil which rapidly solidified. Crystallisation from a mixture of ethyl acetate and light petroleum (b. p. 60—80°) gave 4′-*methoxy*-2 : 6-*dinitrodiphenyl*-4-*carboxylic acid*, m. p. 250—253° (Found: N, 8·8; OMe, 10·3. $C_{14}H_{10}O_7N_2$ requires N, 8·8; OMe, 9·75%).

Methyl 2 : 6-*Diamino*-4′-*methoxydiphenyl*-4-*carboxylate* (XVII; R = CO_2Me).—The above dinitro-compound (20·7 g.) in glacial acetic acid (400 c.c.) was hydrogenated (<1 hr. required) in the presence of palladised charcoal (10%; 2 g.). The solution was filtered, this and subsequent operations being conducted under carbon dioxide. The filtrate was evaporated to dryness under reduced pressure at about 50°. Crystallisation of the residual solid from benzene–light petroleum (b. p. 40—60°) gave the *diamine* (15·6 g., 92%) as brown crystals, m. p. 161—163°. Further crystallisation from the same solvents gave material melting constantly at 166·5—168° but still pale brown (Found: C, 66·2; H, 5·7; N, 10·35. $C_{15}H_{16}O_3N_2$ requires C, 66·1; H, 5·9; N, 10·3%).

The *diacetyl* derivative was prepared by treatment of the diamine (0·12 g.) with acetic anhydride (0·25 g.) in dry pyridine (2 c.c.) at room temperature for 16 hr. The crystals were filtered off, washed with 2N-hydrochloric acid and water, and dried. Crystallisation from a mixture of chloroform and a little light petroleum gave colourless needles, m. p. 253·5—254° (Found: N, 7·7; OMe, 17·0. $C_{19}H_{20}O_5N_2$ requires N, 7·9; OMe, 17·4%).

Methyl 2 : 6-*Di-iodo*-4′-*methoxydiphenyl*-4-*carboxylate* (XVIII; R = CO_2Me).—The foregoing diamine (14·9 g.) was warmed with glacial acetic acid (48 c.c.) and concentrated sulphuric acid (24 c.c.); a little material which remained undissolved was taken up in hot glacial acetic acid and was added to the acetic–sulphuric acid. The solution of the diamine was added, with stirring, at −2° to 0°, during about 90 min. to a solution of nitrosylsulphuric acid prepared from sodium nitrite (9·6 g.), concentrated sulphuric acid (72 c.c.), and acetic acid (144 c.c.). The orange-coloured solution was then stirred at 0° for 1 hr. and added during about 30 min. from an ice-cooled dropping funnel to a vigorously stirred solution of iodine (40 g.), sodium iodide (48 g.), urea (7 g.), and water (750 c.c.) covering a layer of chloroform (400 c.c.). The mixture was stirred overnight at room temperature, the chloroform layer was then separated, and the aqueous layer extracted twice with chloroform. The combined extracts were washed with a concentrated aqueous solution of sodium thiosulphate to remove iodine, then twice with water, and were dried ($CaCl_2$). The solution was evaporated to dryness and the brownish residue was chromatographed in benzene on alumina, with benzene as eluant. The coloured impurities remained near the top of the column while the required *di-iodo*-compound was rapidly eluted. Crystallisation from glacial acetic acid gave colourless prisms (19·9 g.), m. p. 174—176°, and a second crop which, after further crystallisation, yielded a further 0·8 g. of material, m. p. 172—175° (77% in all). Further crystallisation from glacial

acetic acid gave prisms, m. p. 175—176° (Found: C, 36·6; H, 2·3; I, 51·0. $C_{15}H_{12}O_3I_2$ requires C, 36·5; H, 2·45; I, 51·4%).

The corresponding *acid*, prepared by hydrolysis with aqueous-alcoholic sodium hydroxide, separated from aqueous alcohol as colourless needles, m. p. 223—225° (Found: C, 35·3; H, 2·4. $C_{14}H_{10}O_3I_2$ requires C, 35·0; H, 2·1%).

4′-Hydroxy-2 : 6-di-iododiphenyl-4-carboxylic Acid (XXII; X = H).—Methyl *2* : 5-di-iodo-4′-methoxydiphenyl-4-carboxylate (1·0 g.) was boiled under reflux for 2 hr. with constant-boiling hydriodic acid (5 c.c.) and acetic acid (5 c.c.). The ester went partly into solution at first but a light powdery solid soon began to separate. After cooling, the solid was filtered off, washed with water, and dried. After two crystallisations from 50% acetic acid it was obtained in almost theoretical yield as glistening plates, m. p. 268—271° (Found: C, 33·8; H, 2·1; I, 54·9. $C_{13}H_8O_3I_2$ requires C, 33·5; H, 1·7; I, 54·5%).

4′-Hydroxy-2 : 6 : 3′ : 5′-tetra-iododiphenyl-4-carboxylic Acid (XXII; X = I).—4′-Hydroxy-2 : 6-di-iododiphenyl-4-carboxylic acid (0·47 g.) in aqueous ethylamine (20%; 10 c.c.) was shaken while a solution of iodine (0·55 g.) in aqueous sodium iodide was added dropwise. A colourless solid began to separate towards the end of the addition. The mixture was kept for 10 min., the solid was brought into solution by warming, and the solution was acidified to Congo-red with hydrochloric acid. The solid (0·73 g.) crystallised from acetic acid as colourless needles, m. p. 264—265° (decomp.) (Found: C, 22·2; H, 1·1; I, 70·7. $C_{13}H_6O_3I_4$ requires C, 21·75; H, 0·8; I, 70·7%).

4-Acetoxymethyl-2 : 6-di-iodo-4′-methoxydiphenyl (XVIII; R = CH_2·OAc) (*by Reduction of Methyl 2 : 6-Di-iodo-4′-methoxydiphenyl-4-carboxylate*).—The ester (1·0 g., 0·002 mole) in dioxan (distilled over sodium; 15 c.c.) was added fairly rapidly with stirring to ice-cold ethereal lithium aluminium hydride (2·16%; 3·5 c.c.; 0·002 mole). The mixture was then stirred at 0° for 30 min. and decomposed with ice. 2N-Sulphuric acid was added and the solution extracted with chloroform. The extract was washed with water, dried ($MgSO_4$), and evaporated. The residual brown gum (0·88 g.) which did not crystallise, was chromatographed in benzene on a short column of alumina. Benzene eluted only a trace of gum, but 10% of methanol in benzene readily eluted 682 mg. of a gum. This was acetylated by acetic anhydride (1 c.c.) for 1 hr. on the water-bath. Excess of anhydride was destroyed by refluxing ethanol, and the solution was diluted with water and extracted with chloroform. The extract was washed with sodium hydrogen carbonate solution and with water, and was dried ($MgSO_4$). After removal of the solvent the residue was dissolved in boiling light petroleum (b. p. 60—80°), and the solution was decanted from a little gum which separated first. On further cooling, the *acetoxymethyl* compound separated in needles, m. p. 117—119° (Found: C, 38·0; H, 2·7; I, 49·3. $C_{16}H_{14}O_3I_2$ requires C, 37·8; H, 2·8; I, 50·0%).

An experiment, with 1·4 g. (0·00282 mole) of the ester and 0·00168 mole of lithium aluminium hydride gave 0·58 g. of unchanged ester, which was readily eluted by benzene, and, after acetylation, 0·29 g. of the acetoxymethyl compound.

Ethyl α-Acetamido-α-carbethoxy-β-(2 : 6-di-iodo-4′-methoxy-4-diphenylyl)propionate (XIX).—Methyl 2 : 6-di-iodo-4′-methoxydiphenyl-4-carboxylate (5 g.) in dry dioxan (75 c.c.) was reduced as described above, with lithium aluminium hydride in ether (2·2% solution; 13·5 c.c.). Chromatography of the crude product on alumina gave 1·71 g. of unchanged ester, with 2·28 g. of crude alcohol which was eluted with 5% methanol in ether. This crude alcohol in dry benzene (20 c.c.) was treated with thionyl chloride (1·2 c.c.), and the solution was boiled under reflux for 3 hr. The solution was evaporated to dryness under reduced pressure, and the last traces of thionyl chloride were removed by repeated evaporation with benzene.

Sodium (0·12 g.) was dissolved in dry ethanol (5 c.c.), and ethyl acetamidomalonate (1·08 g.) was added. The crude chloro-compound was not very soluble even in hot ethanol but it was added to the sodio-acetamidomalonic ester solution with the help of about 25 c.c. of boiling ethanol. The mixture was boiled under reflux and stirred for 6 hr. A small quantity of solid separated during this time. The hot solution was filtered and evaporated to small bulk. On cooling, a powdery solid separated which was filtered off and washed with ethanol. After crystallisation from aqueous acetic acid the *malonic ester* (0·50 g., 11% based on unrecovered starting material) melted at 205—209° (Found: N, 2·2; I, 37·6. $C_{23}H_{25}O_6NI_2$ requires N, 2·1; I, 38·2%).

4-p-Hydroxyphenyl-3 : 5-di-iodo-DL-phenylalanine (XX).—Ethyl α-acetamido-α-carbethoxy-β-(2 : 6-di-iodo-4′-methoxy-4-diphenylyl)propionate (0·34 g.) was boiled under reflux for 3 hr. with constant-boiling hydriodic acid (2 c.c.) and acetic acid (2 c.c.). The solution was evaporated to dryness, the residual solid dissolved in alcohol, and the solution diluted with

water and brought to pH 5 by addition of sodium acetate solution. On cooling, a powdery solid (0·19 g., 67%) separated, which was filtered off and washed well with water. It decomposed at 196—199° (Found: C, 37·1; H, 3·3; N, 2·6; I, 45·9. $C_{15}H_{13}O_3NI_2,C_2H_6O$ requires C, 36·8; H, 3·4; N, 2·5; I, 45·7%).

4-(4-*Hydroxy*-3 : 5-*di-iodophenyl*)-3 : 5-*di-iodo*-DL-*phenylalanine* (XXI).—4-*p*-Hydroxyphenyl-3 : 5-di-iodophenylalanine (93·5 mg.) in aqueous ethylamine (33%; 1 c.c.) was treated with a solution of iodine in aqueous potassium iodide (1·26N; 0·60 c.c.), gradually with shaking. The iodine was decolorised and towards the end of the addition a white solid started to separate. The mixture was left overnight in the refrigerator, and the solid was then filtered off and suspended in water. Hydrochloric acid (0·2N) was added till the pH was 5 and after some hours the solid was filtered off, washed with water, and dried in a desiccator. It was purified by dissolving it in hot N-sodium hydroxide (1 c.c.), filtering, and adding N-hydrochloric acid to the boiling alkaline solution to bring the pH to 5. After some time in the refrigerator, the nearly colourless solid was filtered off, washed thoroughly with water, and dried at 80°/0·1 mm. The *amino-acid* melted at 202—203° (decomp.) (Found: C, 22·9; H, 1·9; I, 65·2. $C_{15}H_{11}O_3NI_4,H_2O$ requires C, 23·1; H, 1·7; I, 65·2%).

2 : 6-*Diamino*-4-*hydroxymethyl*-4′-*methoxydiphenyl* (XVII; R = CH_2·OH).—A solution of methyl 2 : 6-diamino-4′-methoxydiphenyl-4-carboxylate (6·8 g.) in dry dioxan (250 c.c.) was added to a stirred solution of lithium aluminium hydride in ether (2·28%; 125 c.c.), under nitrogen. The mixture was boiled under reflux for 1 hr., cooled, and decomposed with ice followed by 40% sodium hydroxide solution. The mixture was extracted with chloroform, and the extract washed with water, dried, and evaporated to a brownish gum which crystallised from chloroform–light petroleum (b. p. 60—80°). The *diamine* (4·7 g., 77%) melted at 150—151° after further crystallisation (Found: C, 68·5; H, 6·7; N, 11·45. $C_{14}H_{16}O_2N_2$ requires C, 68·8; H, 6·6; N, 11·5%).

4-*Acetoxymethyl*-2 : 6-*di-iodo*-4′-*methoxydiphenyl* (XVIII; R = CH_2·OAc) (With Dr. J. Attenburrow).—The above diamine (3·4 g.) was tetrazotised in acetic acid (17 c.c.) with sodium nitrite (2·5 g.) in concentrated sulphuric acid (23·5 c.c.) and acetic acid (30 c.c.). The resulting tetrazonium solution was added fairly rapidly to a solution of iodine (9·6 g.), sodium iodide (11·4 g.) and urea (1·42 g.) in water (186 c.c.) covering a layer of chloroform (45 c.c.). The mixture was then stirred for 90 min. and sodium metabisulphite was added, to destroy excess of iodine. The aqueous layer was extracted with chloroform; the combined chloroform solutions were washed with sodium metabisulphite solution and water (formation of emulsions caused some difficulty). The dried solution was evaporated to a brown gum which was chromatographed in benzene on alumina. Elution with benzene gave 4-acetoxymethyl-2 : 6-di-iodo-4′-methoxydiphenyl (2·64 g.) melting at 116—119°. A further quantity (0·86 g.) was obtained by elution with acetone and treatment of the eluate with excess of acetic anhydride on the steam-bath for 1 hr., followed by chromatography of the crude material on alumina. The total yield was 3·5 g., 49%. The m. p. was unchanged after crystallisation of the compound from alcohol (Found: I, 49·6. Calc. for $C_{16}H_{14}O_3I_2$: I, 50·0%).

4-*Hydroxymethyl*-2 : 6-*di-iodo*-4′-*methoxydiphenyl* (XVIII; R = CH_2·OH).—The acetate (7·6 g.) was boiled under reflux for 3 hr. with potassium hydroxide (7·6 g.) in 90% ethanol (76 c.c.). The solution was diluted with water and extracted with ether. The extract was washed with dilute sulphuric acid and evaporated. Crystallisation of the residue from benzene–light petroleum (b. p. 40—60°) gave the *alcohol* (6·1 g., 87%), which melted at 101° after further crystallisation (Found: C, 36·3; H, 2·75; I, 54·0. $C_{14}H_{12}O_2I_2$ requires C, 36·1; H, 2·6; I, 54·5%).

Potassium 4-*Hydroxy*-3 : 5-*dimethylphenyl Sulphate* (XXIII).—Ammonium persulphate (190 g.) in water (2 l.) was added dropwise during 4 hr. to a solution of 2 : 6-dimethylphenol (100 g.) in 4N-potassium hydroxide (1 l.) kept below 20°. Having been stirred overnight the solution was brought to pH 5 with acetic acid, extracted with ether, made alkaline again with potassium hydroxide, and evaporated to dryness at the water pump, and the dark residue was dissolved in boiling alcohol (400 c.c.). On cooling, the solution deposited crystals of the required *potassium* salt. Two further crops were got by concentration of the mother-liquors, giving a total of 156 g. (74%) of crude product. Recrystallisation from alcohol containing a trace of water gave colourless crystals (Found: C, 36·1; H, 3·6; K, 14·6. $C_8H_9O_5SK$ requires C, 37·5; H, 3·5; K, 15·2%).

2 : 6-*Dimethylquinol* (XXIV; R = H).—The crude potassium sulphate (50 g.), dissolved in 2N-hydrochloric acid (250 c.c.), was heated on the steam-bath for 90 min. The cooled solution was twice extracted with ether, and the extract was evaporated. Recrystallisation of

the residue from water (charcoal) gave the quinol (8·0 g., 30%, *i.e.*, 22% overall from the phenol), m. p. 151—152° (Stern *et al.*, *J. Amer. Chem. Soc.*, 1947, **69**, 869, give m. p. 147—149°).

N-*Acetyl*-3′ : 5′-*dimethyl*-3 : 5-*dinitro*-DL-*thyronine Ethyl Ester* (XXV; R = H).—*N*-Acetyl-3 : 5-dinitro-DL-tyrosine ethyl ester (4·2 g.) was heated with toluene-*p*-sulphonyl chloride (2·6 g.) in pyridine (25 c.c.) at 100° for 1 hr. 2 : 6-Dimethylquinol (6·0 g.) was then added, and the mixture was boiled gently for 2 hr. When the mixture was poured into dilute hydrochloric acid a dark gum was precipitated. It was washed with water and extracted with ethyl acetate. After evaporation of the solvent the residue was extracted with a mixture of benzene (100 c.c.) and ethyl acetate (50 c.c.) and the extract was passed down a column of activated alumina. The column was developed with benzene–ethyl acetate (2 : 1) and then ethyl acetate alone. The gums obtained by evaporation of two of the middle fractions showed signs of crystallising. They were therefore dissolved in benzene and chromatographed again. The middle fractions from the second chromatogram partly crystallised. After two crystallisations from ethyl acetate–*cyclo*hexane the *ester* formed golden-yellow blades, m. p. 161° (Found : C, 55·0; H, 5·3; N, 9·1. $C_{21}H_{23}O_9N_3$ requires C, 54·7; H, 5·0; N, 9·1%).

In a second experiment the product was obtained solid at once, but melted over a very wide range and could not be purified by repeated recrystallisation.

4-*Benzyloxy*-3 : 5-*dimethylphenol* (XXIV; R = CH_2Ph).—Benzyl chloride (7 c.c.) was dropped during 2½ hr. into a stirred, boiling solution of potassium 4-hydroxy-3 : 5-dimethylphenyl sulphate (10 g.) and sodium hydroxide (2·8 g.) in water (20 c.c.) and alcohol (25 c.c.). After another 90 min. the solution was made just acid to Congo-red with hydrochloric acid and boiled for 2 hr. more, whereupon a black oil separated from which steam-distillation removed only benzyl alcohol. The aqueous layer was decanted from the tar, which was extracted with ether. The residue from evaporation of the ether was crystallised twice from carbon tetrachloride, to yield the colourless *benzyl ether* (1·6 g., 18%), m. p. 87—88° (Found : C, 78·2; H, 6·9. $C_{15}H_{16}O_2$ requires C, 78·9; H, 7·1%).

N-*Acetyl*-4-(4-*benzyloxy*-3 : 5-*dimethylphenoxy*)-3 : 5-*dinitro*-DL-*phenylalanine Ethyl Ester* (XXV; R = CH_2Ph).—4-Benzyloxy-2 : 6-dimethylphenol (1·35 g., 2 mols.) was added to a solution of *N*-acetyl-3 : 5-dinitro-DL-tyrosine ethyl ester (1·0 g.) and toluene-*p*-sulphonyl chloride (0·62 g., 1·1 mol.) in dry pyridine (25 c.c.) which had been heated on the steam-bath for 30 min. After 90 min.' refluxing the mixture was cooled, diluted with chloroform (100 c.c.), and extracted with dilute hydrochloric acid and sodium carbonate solution. The chloroform was removed under reduced pressure and the residual gum chromatographed in acetone on activated alumina. The yellow fraction which was not adsorbed was evaporated to dryness and the yellow gum crystallised twice from chloroform–*cyclo*hexane. The *diphenyl ether* (1·1 g., 68%) formed pale yellow crystals, m. p. 118—121° (Found : C, 61·2; H, 5·3; N, 7·7. $C_{28}H_{29}O_9N_3$ requires C, 61·0; H, 5·3; N, 7·6%).

3′ : 5′-*Dimethyl*-3 : 5-*dinitro*-DL-*thyronine* (XXVIII).—(*a*) A solution of the above ester (0·5 g.) in ethanol (10 c.c.) and hydrochloric acid (5 c.c.) was heated under reflux for 3½ hr., then concentrated to dryness under reduced pressure, and the residue was dissolved in a little ethanol, the pH being adjusted to 5 by the addition of sodium acetate solution. Addition of water precipitated a yellow solid which, on crystallisation from ethanol, yielded the yellow *amino-acid*, m. p. 203—207° (decomp.) (Found : C, 52·5; H, 4·7; N, 10·1. $C_{17}H_{17}O_8N_3,\frac{1}{2}C_2H_6O$ requires C, 52·2; H, 4·9; N, 10·1%). Dilution of the alcoholic mother-liquor with water yielded a small quantity of yellow solid, m. p. 163°, probably the *O*-benzyl compound.

(*b*) A mixture of *N*-acetyl-3′ : 5′-dimethyl-3 : 5-dinitro-DL-thyronine ethyl ester (0·2 g.), ethanol (2 c.c.), concentrated hydrochloric acid (1 c.c.), and water (1 c.c.) was boiled under reflux for 2 hr. Most of the ethanol was removed by distillation and the residue was allowed to cool, whereupon an oil separated which later solidified. It recrystallised from dilute hydrochloric acid as a mixture of yellowish prisms and fine colourless needles, the latter in very small amount. It was possible to remove the needles by decantation. The residual crystals were covered with water, and 2N-sodium hydroxide was added with shaking until the solid had completely dissolved. On addition of hydrochloric acid to the dark solution, fine yellow needles started to be precipitated as soon as the colour had disappeared. Excess of hydrochloric acid was added and the solid was filtered off and recrystallised from water. The orange-yellow crystals darkened on heating at about 200° and melted with decomposition at 216°.

A mixture of the compounds prepared by methods (*a*) and (*b*) melted between the m. p.s of the constituents.

4-*Methoxy*-3 : 5-*dimethylphenol* (XXIV; R = Me).—A mixture of potassium 4-hydroxy-

3 : 5-dimethylphenyl sulphate (20 g.), 90% alcohol (320 c.c.) and methyl sulphate (80 c.c.) was stirred and cooled while 40% sodium hydroxide (250 c.c.) was added during 1 hr. Next morning the mixture was heated on the steam-bath for 30 min., cooled, acidified with concentrated hydrochloric acid, and re-heated for 30 min. Steam-distillation produced a milky distillate which was extracted with ether. Evaporation of the ether left the methoxy-compound (4·5 g., 38%), which melted at 83° after recrystallisation from water (Baker and Brown, *J.*, 1948, 2303, give m. p. 83°).

N-*Acetyl*-4-(4-*methoxy*-3 : 5-*dimethylphenoxy*)-3 : 5-*dinitro*-DL-*phenylalanine Ethyl Ester* (XXV; R = Me).—4-Methoxy-3 : 5-dimethylphenol (1·2 g.) was added to a solution of *N*-acetyl-3 : 5-dinitro-DL-tyrosine ethyl ester (1·0 g.) and toluene-*p*-sulphonyl chloride (0·62 g.) in pyridine (25 c.c.) which had been heated on the steam-bath for 30 min. When it had been boiled for 2 hr. the mixture was poured into dilute hydrochloric acid and extracted with chloroform. The gum remaining from evaporation of the chloroform was dissolved in acetone and passed down a column of alumina. The pale yellow solution which passed straight through was evaporated and the residue was recrystallised twice from aqueous alcohol. The *diphenyl ether* (0·89 g., 53%) was obtained as yellow crystals, m. p. 116—120° (Found: C, 55·9; H, 5·4; N, 8·5. $C_{22}H_{25}O_9N_3$ requires C, 55·6; H, 5·3; N, 8·8%).

N-*Acetyl*-3 : 5-*di-iodo*-4-(4-*methoxy*-3 : 5-*dimethylphenoxy*)-DL-*phenylalanine Ethyl Ester* (XXVI; R = Me).—The dinitro-compound (1·0 g.) in acetic acid (50 c.c.) was hydrogenated in the presence of palladised charcoal (0·5 g.) at room temperature and pressure. After 40 hr. the uptake of hydrogen was almost theoretical. Sulphuric acid (20 c.c.) was gradually added to the solution with cooling, and the resulting mixture was added during 45 min. to a solution of sodium nitrite (0·35 g.; 2·4 mols.) in sulphuric acid (50 c.c.) and acetic acid (50 c.c.) kept at −5°. After 90 min. this tetrazonium solution was run into a stirred mixture of water (300 c.c.) and chloroform (150 c.c.) containing iodine (5 g.) and sodium iodide (5 g.). Half an hour later the chloroform was separated, washed with sodium metabisulphite, and then water, and evaporated to dryness. The residue, in acetone, was passed down a column of activated alumina. Evaporation of the solvent left a yellow gum, that solidified when scratched under light petroleum. The *di-iodo*-compound (0·62 g., 46%) formed pale buff crystals, melting at 149—151° after recrystallisation from aqueous alcohol (Found: C, 41·4; H, 4·2; I, 39·1. $C_{22}H_{25}O_5NI_2$ requires C, 41·4; H, 3·9; I, 39·9%).

3 : 5-*Di-iodo*-3′ : 5′-*dimethyl*-DL-*thyronine* (XXVII).—A solution of the acetamido-ester (1·8 g.) in acetic acid (12 c.c.) and constant-boiling hydriodic acid (8 c.c.) was boiled for 3½ hr. The residue left after removal of the solvents under reduced pressure was dissolved in boiling alcohol containing a trace of sodium metabisulphite, and sodium acetate solution was added until the pH reached 5. The gelatinous precipitate was filtered off and washed thoroughly with water. It was redissolved in alcoholic hydrochloric acid and brought out of solution again with sodium acetate, to give the colourless *amino-acid* (1·2 g., 75%), m. p. 230—233° (decomp.) (Found: C, 35·9; H, 3·5; I, 44·6. $C_{17}H_{17}O_4NI_2,H_2O$ requires C, 35·7; H, 3·3; I, 44·45%).

After only 2 hr. boiling a compound melting at about 160° could be isolated from the mother-liquors after removal of the above amino-acid. Since it gave the amino-acid on further boiling with hydriodic and acetic acids, it must have been the *O*-methyl-amino-acid.

N-*Acetyl*-3 : 5-*dicyano*-4-p-*methoxyphenoxy*-L-*phenylalanine Ethyl Ester* (XXIX; R = CN).—A solution of *N*-acetyl-3 : 5-di-iodo-4-*p*-methoxyphenoxy-L-phenylalanine ethyl ester (50 g.) in pyridine (75 c.c.) containing cuprous cyanide (25 g.) was refluxed for 6 hr. The semi-solid mass was poured into water, and the solid was filtered off and washed with water. It was then added to 2N-ammonia (500 c.c.) and chloroform (300 c.c.), and the mixture was stirred thoroughly. After filtration of the mixture through kieselguhr, the chloroform layer was separated, washed successively with 2N-ammonia, 2N-hydrochloric acid, and water, and was dried (K_2CO_3). The solvent was removed and the residue crystallised from ethanol (charcoal). The *dicyano*-compound (27 g., 81%) had m. p. 139—140° and $[\alpha]_D$ +58° (*c*, 1·24 in $CHCl_3$) (Found: C, 64·6; H, 5·4; N, 10·1. $C_{22}H_{21}O_5N_3$ requires C, 64·9; H, 5·2; N, 10·3%).

3 : 5-*Dicarboxy*-L-*thyronine*.—A solution of *N*-acetyl-3 : 5-dicyano-4-*p*-methoxyphenoxy-L-phenylalanine ethyl ester (5 g.) in boiling hydriodic acid (*d* 1·7; 50 c.c.) was refluxed for 6 hr. The solvent was then distilled off and the residue was dissolved in 2N-sodium carbonate. Addition of 2N-hydrochloric acid precipitated a solid which was filtered off, washed, and purified by reprecipitation. The *acid* did not melt at 360° and was not soluble in any of the usual solvents. It had $[\alpha]_D^{24}$ +19° (*c*, 1·06 in 2N-Na_2CO_3) (Found: C, 56·3; H, 4·0; N, 3·7. $C_{17}H_{15}O_8N$ requires C, 56·5; H, 4·2; N, 3·9%).

3-*Iodo*-2-p-*methoxyphenoxy*-5-*methylbenzoic acid* (XXX; R = CO_2H).—Magnesium (0·36 g.) was added to a solution of 2 : 6-di-iodo-4′-methoxy-4-methyldiphenyl ether (Part VIII, *J.*, 1951, 2467) (3·3 g.) in dry ether (40 c.c.). Attempts to start the reaction were unsuccessful, so a further quantity of magnesium (0·24 g.) was added and then methyl iodide (1·42 g.) in ether (14 c.c.) was run slowly on to the mixture. The mixture was gently refluxed for 24 hr., most of the magnesium dissolving. The product was poured on an excess of solid carbon dioxide, and the mixture set aside, then decomposed with ice and dilute hydrochloric acid, and extracted with ether. Extraction of the ethereal solution with sodium hydrogen carbonate solution, followed by acidification, gave the crude *acid* (1·2 g., 44%) which, after crystallisation from aqueous acetic acid and from *cyclo*hexane, was obtained as colourless needles, m. p. 184—185° (Found: C, 47·5; H, 3·75; I, 32·9. $C_{15}H_{13}O_4I$ requires C, 46·9; H, 3·4; I, 33·0%).

4′-*Methoxy*-2 : 6-*dimethyldiphenyl ether* (XXXI) (With MR. F. F. STEPHENS).—*p*-Iodoanisole (11·7 g.), 2 : 6-dimethylphenol (6·1 g.), copper bronze powder (0·1 g.), and anhydrous potassium carbonate (6·9 g.) were refluxed for 7 hr. The internal temperature was initially 216° and rose during the reaction to 242°. After cooling, the solid product was diluted with water and extracted with ether, and the extract washed with 2N-sodium hydroxide and water and evaporated. The residual dark brown oil was dissolved in ether and the solution passed down a column of alumina, which removed some dark brown material. The residue after evaporation of the ether was distilled and the fraction boiling between 155° and 193° at 14—16 mm. was collected. This fraction (6 g.) crystallised overnight and melted at 41—42° (after removal of a little oil on a porous tile). An analytical sample was obtained by fractional steam-distillation, followed by low-temperature crystallisation of the less volatile fraction from ether. The *diphenyl ether* was finally obtained as a colourless solid, m. p. 41·5—42·5° (Found: C, 78·8; H, 6·95. $C_{15}H_{16}O_2$ requires C, 78·9; H, 7·1%).

N-*Acetyl*-L-*tyrosine Ethyl Ester*.—A mixture of *N*-acetyl-L-tyrosine (du Vigneaud and Meyer, *J. Biol. Chem.*, 1932, **98**, 295) (20 g.), toluene-*p*-sulphonic acid (2 g.), ethanol (20 c.c.), and chloroform (500 c.c.) was boiled under reflux for 8 hr., the water formed in the reaction being removed azeotropically. The tyrosine derivative gradually dissolved. The resultant solution was washed with aqueous sodium hydrogen carbonate and water and was evaporated to dryness under reduced pressure. The residue was crystallised from aqueous methanol, giving colourless crystals of the ester monohydrate (21·5 g.; 89%), m. p. 84—87°, $[\alpha]_D^{20}$ +24° (*c*, 1 in EtOH) (Found, in material dried *in vacuo* at room temperature: C, 57·7; H, 7·0; N, 5·1. Calc. for $C_{13}H_{17}O_4N,H_2O$: C, 58·0; H, 7·1; N, 5·2%). A portion of the material was dried at 100° *in vacuo* (Found: C, 61·7; H, 6·7; N, 5·5. Calc. for $C_{13}H_{17}O_4N$: C, 62·1; H, 6·8; N, 5·6%). Thomas, MacAllister, and Niemann (*J. Amer. Chem. Soc.*, 1951, **73**, 1548) give m. p. 96—97°, $[\alpha]_D^{25}$ +24·7°; Kaufman, Neurath, and Schwert (*J. Biol. Chem.*, 1949, **177**, 793) give m. p. 79—80°. The degree of hydration is not specified in either publication.

N-*Acetyl*-4-(4-*methoxy*-2 : 6-*dinitrophenoxy*)-L-*phenylalanine Ethyl Ester* (XXXIII).—A solution of 1-(2 : 6-dinitro-4-methoxyphenyl)pyridinium toluene-*p*-sulphonate (Part VII, *J.*, 1950, 2824) (24·5 g., 1 mol.) and *N*-acetyl-L-tyrosine ethyl ester monohydrate (27·5 g., 2 mol.) in pyridine (250 c.c.) was boiled under reflux for 90 min., then cooled and poured into dilute hydrochloric acid containing ice. The resulting gum was taken up in chloroform and the solution washed successively with 2N-hydrochloric acid, 2N-sodium hydroxide, and water. The solution was concentrated and passed through a short column of alumina; the product passed through rapidly and was obtained crystalline by evaporation. The *diphenyl ether* (14·8 g., 60%), obtained as yellow needles by recrystallisation from aqueous ethanol, had m. p. 134—135°, $[\alpha]_D^{20}$ +64·1 (*c*, 2·2 in $CHCl_3$) (Found: C, 53·95; H, 4·7; N, 9·2. $C_{20}H_{21}O_9N_3$ requires C, 53·7; H, 4·7; N, 9·4%). This material, after 2 weeks' storage, had m. p. 160—161°, and a later batch consisted of the higher-melting form.

When one molar proportion of the tyrosine derivative was employed, the yield was 37%.

N-*Acetyl*-4-(2 : 6-*diamino*-4-*methoxyphenoxy*)-L-*phenylalanine Ethyl Ester*.—The foregoing dinitro-compound (14·7 g.) in ethanol (750 c.c.) was hydrogenated at 90°/142 atm. for 3 hr., in the presence of palladised charcoal (3 g.). The suspension was filtered in an atmosphere of carbon dioxide and evaporated at <40° under reduced pressure, to an almost colourless syrup (12·0 g., 94%).

The *diacetyl* derivative, prepared by dissolving the diamine in boiling acetic anhydride and leaving the solution overnight at room temperature, separated from ethanol as colourless needles, m. p. 193°, $[\alpha]_D^{20}$ +53·8° (*c*, 2 in $CHCl_3$) (Found: C, 61·25; H, 6·5; N, 8·9. $C_{24}H_{29}O_7N_3$ requires C, 61·1; H, 6·2; N, 8·9%).

N-*Acetyl*-4-(2 : 6-*di-iodo*-4-*methoxyphenoxy*)-L-*phenylalanine Ethyl Ester* (XXXIV).—The

above diamine (8·0 g.) was dissolved in glacial acetic acid (80 c.c.) and concentrated sulphuric acid (15 c.c.), and the solution was added dropwise at 0° during 45 min. to a stirred solution of nitrosylsulphuric acid prepared from sodium nitrite (3·25 g.), sulphuric acid (30 c.c.), and acetic acid (25 c.c.) in the usual way. After a further hr. stirring the tetrazonium solution was added to a well-stirred mixture of sodium iodide (18·9 g.), iodine (16·8 g.), urea (3 g.), water (300 c.c.), and chloroform (300 c.c.). After being stirred for 2 hours the mixture was filtered from free iodine, and the chloroform layer was separated and washed successively with sodium metabisulphite solution, sodium hydrogen carbonate solution, and water. The chloroform solution was evaporated and the residue was passed, in chloroform, through a short alumina column. The desired product passed through rapidly and was recovered by evaporation. Crystallisation of this material from ethanol (charcoal) afforded the *di-iodo*-compound (6·8 g., 52%) as colourless needles, m. p. 171·5—172·5°, $[\alpha]_D^{19}$ +48·0° (*c*, 2 in $CHCl_3$) (Found: C, 39·3; H, 3·6; N, 2·3; I, 41·4. $C_{20}H_{21}O_5NI_2$ requires C, 39·4; H, 3·5; N, 2·3; I, 41·7%).

2′ : 6′-*Di-iodo*-L-*thyronine* (XXXV).—The above product (2 g.) in hydriodic acid (57%; 5 c.c.) and glacial acetic acid (5 c.c.) was boiled under reflux for 4 hr., and the solution evaporated to dryness under reduced pressure. The residue was taken up in hot water containing a little hydrochloric acid, and sodium metabisulphite was added to remove free iodine. Hot sodium acetate solution was then added until the pH was 4—5; a finely divided solid was precipitated. This was dissolved in hot 80% ethanol containing a little sodium hydroxide, and precipitated by adjustment of the pH to 4—5 by hot 0·5N-hydrochloric acid. The *amino-acid* (1·6 g., 93%), a nearly colourless powder, had m. p. 245—246° (decomp.) and $[\alpha]_D^{21}$ +6·5° [*c*, 1·9 in 1 : 1 (v/v) of N-HCl–EtOH] (Found: C, 34·5; H, 2·9; N, 2·7; I, 48·1. $C_{15}H_{13}O_4NI_2$ requires C, 34·3; H, 2·5; N, 2·7; I, 48·3%).

N-*Acetyl*-4-(2 : 6-*di-iodo*-4-*methoxyphenoxy*)-DL-*phenylalanine Ethyl Ester* (XXXIV).—Sodium (0·126 g.) was dissolved in dry ethanol (100 c.c.), *N*-acetyl-4-(2 : 6-di-iodo-4-methoxyphenoxy)-L-phenylalanine ethyl ester (3·05 g.) was added, and the solution was boiled under reflux for 3 hr. Chloroform (300 c.c.) was then added and the mixture, stirred and cooled in ice, was treated with 0·5N-hydrochloric acid, during a few min., until a faintly acid reaction was observed. Water was added and the chloroform layer was quickly separated and shaken briefly with N-sodium carbonate solution, and then with water. The chloroform layer, on evaporation under reduced pressure, afforded the desired *ester* (1·6 g., 52%) which was obtained as needles, m. p. 149—151°, by repeated recrystallisation from ethanol (Found: C, 39·3; H, 3·6; I, 42·15. $C_{20}H_{21}O_5NI_2$ requires C, 39·4; H, 3·5; I, 41·7%). The material was optically inactive.

2′ : 6′-*Di-iodo*-DL-*thyronine* (XXXV)—This material was obtained by hydrolysis of the foregoing DL-acetamido-ester by the method employed for the L-form. After repeated reprecipitation a nearly colourless powder was obtained, having m. p. 235° (decomp.). This material was optically inactive (Found: C, 34·35; H, 2·95; N, 2·5; I, 47·2. Calc. for $C_{15}H_{13}O_4NI_2$: C, 34·3; H, 2·5; N, 2·7; I, 48·3%). Niemann and McCasland (*J. Amer. Chem. Soc.*, 1944, **66**, 1870) give m. p. 220—221°.

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