Synthesis of some Possible Metabolites of Thyroxine and Triiodothyronine

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Interest in the acetic acid analogues* of thyroxine [3:5-diiodo-4-(4'-hydroxy-3':5'-diiodophenoxy)-phenylacetic acid] and triiodothyronine [3:5-diiodo-4-(4'-hydroxy-3'-iodophenoxy)phenylacetic acid] has been stimulated by the observations of Thibault & Pitt-Rivers (1955), who demonstrated that both substances produced an immediate rise in the rate of oxygen consumption of rat-kidney slices and, at certain dose levels, in the basal metabolic rate of thyroidectomized rats. When tested in rats both substances had previously been shown to exert thyroxine-like activity, results which led Pitt-Rivers (1953) to suggest that there is strong presumptive evidence that these compounds may play a part in the metabolism of the thyroid hormone. Trotter (1955) showed that triiodothyroacetic acid produced a beneficial effect in human myxoedema, though attempts to demonstrate an immediate rise in the basal metabolic rate were inconclusive.

As a supply of these substances was required for investigation by a number of techniques applied to other analogues of thyroid hormones in these laboratories, a study has been made of their methods of preparation.

Diiodothyroacetic acid and the tetraiodo-compound were originally prepared by Harington & Pitt-Rivers (1952), and Pitt-Rivers (1953) subsequently obtained the triiodo compound by partial iodination of the diiodo derivative. The starting material used by these authors was the relatively inaccessible 3:5-diiodo-4-(4'-methoxyphenoxy)benzaldehyde, but the synthesis of thyroxine by Borrows, Clayton & Hems (1949) suggested a more convenient preparative route for the required compounds.

p-Hydroxyphenylacetic acid was nitrated and the dinitro-derivative converted into its ethyl ester (I). The toluene-*p*-sulphonyl derivative of the ester reacted in anhydrous pyridine with *p*-methoxyphenol under conditions similar to those used by Borrows, Clayton, Hems & Long (1949) to give ethyl 3:5-dinitro-4-(4'-methoxyphenoxy)phenylacetate (II). Catalytic reduction followed by tetrazotization of the resulting diamine and reaction with aqueous sodium iodide gave ethyl 3:5-diiodo-4-(4'-methoxyphenoxy)phenylacetate (III) in good yield. Concurrent hydrolysis and demethylation of the latter with hydriodic acid gave the required diiodothyroacetic acid (IV), which on iodination produced the triiodo- (V) and tetraiodo- (VI) compounds.

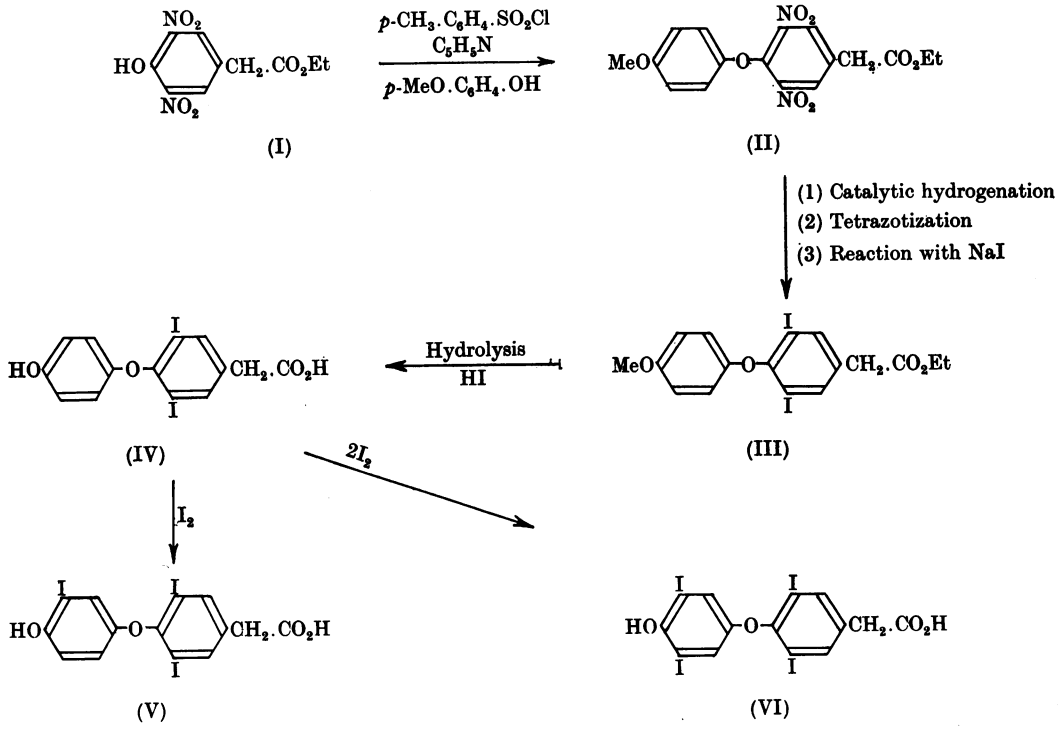
A preliminary account of this synthesis has already appeared (Wilkinson, 1955).

Recent studies of the deiodination of thyroxine and triiodothyronine in liver homogenates (Maclagan & Sprott, 1954; Sprott & Maclagan, 1955; Maclagan & Reid, 1955) have shown that an aerobic system is involved. This implies that the deiodination may be hydrolytic rather than reductive in nature. If this is so then the first product from thyroxine would be 3'-hydroxy-3:5:5'-triiodo-L-thyronine and, from triiodothyronine, 3'-hydroxy-3:5-diiodo-L-thyronine. Attempts have therefore been made to synthesize these two compounds so that their biological properties could be investigated.

A route similar to that outlined above was employed. 3:5-Dinitro-*N*-acetyl-L-tyrosine ethyl ester was condensed with 3:4-methylenedioxyphenol (sesamol) to give 3:5-dinitro-4-(3':4'-methylenedioxyphenoxy)phenyl-*N*-acetyl-L-alanine ethyl ester, which was reduced and the resulting diamine tetrazotized. The tetrazonium salt reacted with sodium iodide to give the corresponding 3:5-diiodo compound. This material proved difficult to hydrolyse completely for, although it was easily saponified to 3:5-diiodo-4-(3':4'-methylenedioxyphenoxy)-phenyl-*N*-acetyl-L-alanine, attempts to remove the acetyl group by heating with mineral acids gave brownish products which could not be purified. Similar difficulties were experienced when attempts were made to remove the protecting methylene group at the same time.

The results of the biological investigations will be reported later.

* The term thyroacetic acid has been suggested by Pitt-Rivers (1953) for the 4-(4'-hydroxyphenoxy)phenylacetic acid system of these compounds. For convenience this term is used where appropriate throughout this paper. The trivial names triac and tetrac have been applied to the 3:5:3'-triiodo and 3:5:3':5'-tetraiodo derivatives respectively (Trotter, 1955; Heimberg, Park, Isaacs & Pitt-Rivers, 1955).



EXPERIMENTAL

Synthetic methods

Melting points are not corrected. Micro-analyses were by the Organic Micro-analytical Laboratory of Imperial College.

p-*Hydroxyphenylacetic acid.* *p*-Nitrophenylacetic acid (Hopkin & Williams, Ltd., London) was catalytically reduced to the corresponding *p*-amino compound (Ferber & Bendix, 1939). The latter (11·7 g.) was dissolved in hot 4N-H_2SO_4 (200 ml.) and cooled. The resulting suspension of the sulphate was diazotized at 0° by the gradual addition of a solution of $NaNO_2$ (5·35 g.) in water (15 ml.) over 1 hr. The mixture was kept at 0° for a further 1 hr., then run into mechanically stirred boiling 2N-H_2SO_4 (200 ml.) at such a rate that the temperature was kept at 85–95°. Boiling and stirring were continued for a further 45 min., after which a small amount of gummy material was removed. The cooled solution was extracted several times with ether and the combined ether layer extracted three times with 2N-NaOH (50 ml., 2 × 25 ml.). The alkaline solution was acidified with 2N-HCl and the product extracted into ether. The ethereal solution was dried (Na_2SO_4) and the solvent removed. Yield: 10·7 g. (91%); m.p. 146–147°. After crystallization from water the product melted at 148°, in agreement with the figures in the literature.

4-*Hydroxy*-3:5-*dinitrophenylacetic acid.* Nitric acid (sp.gr. 1·42, 11·5 ml.) was added dropwise to a mechanically stirred solution of *p*-hydroxyphenylacetic acid (8·56 g.) in acetic acid (32 ml.). The temp. was initially maintained at 20–25° by external cooling but, when about half the HNO_3 had been added, the temperature was allowed to rise to 45–50° and was maintained in that range until the addition was complete. Excessive heating resulted in partial oxidation with consequent diminution in yield. After the addition, which required about 2 hr., was completed, the mixture was kept at 45–50° for 30 min. The volatile acids were removed under reduced pressure and the crystalline residue was treated with water (60 ml.). The product was collected by filtration, washed twice with water (20 ml.) and dried at 20°/3 mm. over NaOH and silica gel. Yield: 10·6 g. (78%). After crystallization from water, the *dinitro acid* formed bright-yellow needles, m.p. 176–177°. (Found: C, 39·9; H, 2·8; N, 11·8. $C_8H_6O_7N_2$ requires C, 39·7; H, 2·5; N, 11·6%.)

When H_2SO_4 was used in place of acetic acid and the excess acids were neutralized with NaOH, the main product was a tar from which only small yields of the required acid could be isolated. Treatment of the tar with acetone gave a small quantity of a crystalline solid which appeared to consist of the mono-sodium salt of 5-carboxymethyl-2-hydroxy-3-nitrobenzenesulphonic acid. (Found: C, 31·1; H, 2·9; N, 5·5; S, 10·0. $C_8H_6O_8NSNa$ requires C, 32·1; H, 2·0; N, 4·7; S, 10·6%.)

Ethyl 4-*hydroxy*-3:5-*dinitrophenylacetate* (I). The dinitro acid (9·63 g.) was suspended in dry $CHCl_3$ (300 ml.) containing ethanol (50 ml.) and toluene-*p*-sulphonic acid (1 g.). The mixture was heated under reflux in an apparatus designed for the separation of water. After 4 hr. a clear solution was obtained and the theoretical amount of water had separated. The solution was cooled to about 30° and extracted with N-Na_2CO_3. When the temperature was allowed to fall below 25° the sparingly soluble sodium salt was liable to crystallize. Immediately after separation the alkaline layer was acidified with HCl to precipitate the

required ester. The gum which separated crystallized on trituration. The solid (10·0 g., 93%) was washed with water and dried over silica gel at 20°/3 mm. The *ester* crystallized from ethanol in pale-yellow needles, m.p. 71°. (Found: C, 44·5; H, 3·8; N, 10·4. $C_{10}H_{10}O_7N_2$ requires C, 44·5; H, 3·7; N, 10·4%.)

Ethyl 3:5-*dinitro*-4-(4′-*methoxyphenoxy*)*phenylacetate* (II). A mixture of ethyl 4-hydroxy-3:5-dinitrophenylacetate (9·5 g.), toluene-*p*-sulphonyl chloride (7·0 g.) and anhydrous pyridine (12 ml.) was heated at 95–100° for 30 min. *p*-Methoxyphenol (8·4 g.) was then added and the bath temperature raised to 125±5° for 2 hr. Throughout this period N_2 was passed through the mixture. After the bulk of the pyridine had been removed by distillation under reduced pressure, the residue was dissolved in acetone (40 ml.) and the solution passed through a column of Al_2O_3 (15 cm. × 2 cm.). Acetone was passed through the column until the eluate was almost colourless. The combined eluate was evaporated to dryness and the residue triturated with benzene (150 ml.) and N-KOH (100 ml.). The aqueous layer was discarded and the benzene layer washed successively with N-KOH, N-HCl and water. After filtration to remove a small amount of solid material, the benzene solution was concentrated to about 50 ml. and passed through a column of Al_2O_3 (20 cm. × 2 cm.). The column was eluted with benzene and the yellow eluate evaporated to dryness. The residue which crystallized on standing proved to consist of the required diphenyl ether (9·85 g., 75%). Recrystallization from ethanol gave yellow prisms of *ethyl* 3:5-*dinitro*-4-(4′-*methoxyphenoxy*)*phenylacetate*, m.p. 76°. (Found: C, 54·1; H, 4·35; N, 7·8. $C_{17}H_{16}O_8N_2$ requires C, 54·3; H, 4·25; N, 7·45%.)

Ethyl 3:5-*diamino*-4-(4′-*methoxyphenoxy*)*phenylacetate*. A solution of the dinitro ester (12·7 g.) in ethanol (250 ml.) was hydrogenated at 1 atm. with 5% Pd–$CaCO_3$ catalyst (5 g.). The H_2 absorbed was 103% of the theoretical amount. The catalyst was removed by filtration and the solvent evaporated, these and all subsequent operations being conducted as far as possible in an atmosphere of N_2. The resulting diamine (yield: about theoretical) failed to crystallize and rapidly discoloured on exposure to air. The product was therefore characterized by heating a sample at 60–70° with acetic anhydride to form the *diacetyl derivative*, which crystallized from ethanol in colourless needles, m.p. 204–205°. (Found: C, 63·0; H, 6·0; N, 7·3. $C_{21}H_{24}O_6N_2$ requires C, 63·0; H, 6·0; N, 7·0%.)

Ethyl 3:5-*diiodo*-4-(4′-*methoxyphenoxy*)*phenylacetate* (III). The diamino ester (10·6 g.) was dissolved in a mixture of H_2SO_4 (sp.gr. 1·84) (30 ml.) and acetic acid (30 ml.), and tetrazotized at 0±2° with a solution of $NaNO_2$ (4·64 g.) in H_2SO_4 (sp.gr. 1·84) (40 ml.) and acetic acid (40 ml.). The addition of the nitrite solution occupied about 45 min. After standing for a further 1 hr. at 0°, the tetrazo solution was added over 5 min. to a mixture of a solution of NaI (20 g.), I_2 (10 g.) and urea (4 g.) in water (300 ml.) and $CHCl_3$ (100 ml.), mechanical stirring being employed throughout the addition and for 1 hr. subsequently. The $CHCl_3$ layer was separated and washed free from I_2 with 10% (w/v) $Na_2S_2O_5$, then with water. The solvent was removed and the gummy residue purified by chromatography. A solution in benzene (20 ml.) was applied to a column of Al_2O_3 (Hopkin & Williams Ltd. 'Activated, for Chromatographic Analysis') (22 cm. × 2 cm.) which was eluted with the same solvent. The eluate was collected in three fractions. The first two of 70 and 100 ml. gave 0·22 and 12·2 g. respectively of the *diiodo ester* on evaporation (total yield: 69%). The third fraction (100 ml.), on evaporation, gave a pale-yellow oil (0·17 g.) which could not be induced to crystallize. The first two fractions were combined and recrystallized from ethanol. The product was obtained as colourless needles, m.p. 87°. (Found: C, 37·8; H, 3·1; I, 46·7. $C_{17}H_{16}O_4I_2$ requires C, 38·0; H, 3·0; I, 47·2%.)

3:5-*Diiodo*-4-(4′-*methoxyphenoxy*)*phenylacetic acid*. The ethyl ester (0·295 g.) was hydrolysed by boiling under reflux with N-NaOH (5 ml.) for 45 min. The cooled solution was acidified with HCl to precipitate the *acid*, which was purified by solution in 0·1 N-Na_2CO_3 and precipitation with HCl. Yield: 0·240 g. (86%). It crystallized from 60% (v/v) aqueous ethanol in colourless prisms, m.p. 161–162°. (Found: C, 35·8; H, 2·8; I, 49·2. $C_{15}H_{12}O_4I_2$ requires C, 35·3; H, 2·4; I, 49·8%.)

3:5-*Diiodo*-4-(4′-*hydroxyphenoxy*)*phenylacetic acid* (IV). A solution of ethyl 3:5-diiodo-4-(4′-methoxyphenoxy)-phenylacetate (9·5 g.) in acetic acid (60 ml.) was heated under reflux with HI (sp.gr. 1·7, 50 ml.) and red P (0·5 g.) for 1 hr. The hot solution was filtered and the filtrate concentrated at 50°/15 mm. to about 20 ml. The residue was treated with water (70 ml.) containing a little $Na_2S_2O_5$ to decolorize the product. The solid was collected by filtration and purified by the method of Harington & Pitt-Rivers (1952). Yield: 8·36 g. (95%). After crystallization from 70% (v/v) acetic acid it melted at 219°. Harington & Pitt-Rivers (1952) report m.p. 214–216·5°. (Found: C, 33·9; H, 2·3; I, 51·2. Calc. for $C_{14}H_{10}O_4I_2$: C, 33·9; H, 2·0; I, 51·2%.)

3:5-*Diiodo*-4-(3′:5′-*diiodo*-4′-*hydroxyphenoxy*)*phenylacetic acid* (VI). This was obtained in 93% yield when the diiodo acid was iodinated by a method similar to that of Harington & Pitt-Rivers (1952). The product, crystallized from 60% (v/v) methanol and dried at 25°/3 mm. over silica gel, proved to be an alcoholate whose melting point depended on the rate of heating: 212° (decomp.) (slow), 219–220° (decomp.) (rapid). Harington & Pitt-Rivers reported 219–220° (decomp.). (Found: C, 23·7; H, 1·8; I, 65·0; loss in weight at 100°/3 mm., 4·1. $C_{14}H_8O_4I_4,CH_3.OH$ requires C, 23·1; H, 1·54; I, 65·1; $CH_3.OH$, 4·1%). When heated to constant weight at 100°/3 mm. the product gave the following figures on analysis. (Found: C, 23·0; H, 1·3; I, 67·0. Calc. for $C_{14}H_8O_4I_4$: C, 22·4; H, 1·1; I, 67·9%.) Tetraiodothyroacetic acid proved to be somewhat unstable when dissolved in either ethanolic or alkaline solutions, especially when heated. Oxidation appeared to occur with the formation of a brownish material insoluble in methanol.

3:5-*Diiodo*-4-(3′-*iodo*-4′-*hydroxyphenoxy*)*phenylacetic acid* (V). When the diiodo acid was iodinated as described by Pitt-Rivers (1953) the triiodo compound was obtained in 81% yield. After crystallization from 50% (v/v) methanol, triiodothyroacetic acid was obtained as colourless needles which melted over the range 65–90°, according to the rate of heating. The molten form resolidified at about 110° and finally melted at 180–181° without decomposition. Pitt-Rivers reported that her material had an indeterminate m.p. in the neighbourhood of 100°. The compound, dried at 25°/3 mm. over silica gel, contains methanol of crystallization. (Found: C, 27·0; H, 2·3; I, 60·2; loss in weight at 100°/3 mm., 2·2. $C_{14}H_9O_4I_3$, $\frac{1}{2}CH_3.OH$ requires C, 27·3; H, 1·7; I, 59·8; $CH_3.OH$, 2·5%.) When dried at 100°/3 mm., the following analytical figures were obtained. (Found: C, 27·2;

H, 1·8; I, 61·9. Calc. for $C_{14}H_9O_4I_2$: C, 27·0; H, 1·45; I, 61·2%.)

3:4-*Methylenedioxyphenol (sesamol)*. This was prepared by the oxidation of piperonal with peracetic acid substantially as described by Böeseken, Cohen & Kip (1936). The yield of 60% obtained by these authors could not be reproduced, the best yield in these laboratories being 38%. The method of isolation was therefore modified. The crude product (from 75 g. of piperonal) in ethereal solution was extracted into N-NaOH. After washing with ether the alkaline solution was acidified with HCl and the product extracted into ether. The extract was dried (Na_2SO_4) and the solvent removed. The residue, on distillation at 90° (bath temp.)/10^{-4} mm., gave the required phenol (31·6 g., 46%), m.p. 63–65°. Böeseken *et al.* (1936) report m.p. 65·8°. A sample for analysis was further purified by sublimation at 72°/10^{-4} mm., followed by crystallization from light petroleum (b.p. 60–80°). It formed needles, m.p. 64·5°, which remained colourless when exposed to air for several months. Böeseken *et al.* stated that their product became pink on storage. (Found: C, 60·8; H, 4·5. Calc. for $C_7H_6O_3$: C, 60·8; H, 4·35%.)

The *toluene*-p-*sulphonate* was prepared in 82% yield by shaking a solution of sesamol (0·69 g.) in 2N-NaOH (2·75 ml.) with toluene-*p*-sulphonyl chloride (1·05 g.) at 60° for 30 min. Excess of NaOH was then added and the mixture shaken at 20° for a further 30 min. to decompose unreacted sulphonyl chloride. The ester was collected by filtration, washed with water, and dried over silica gel. It crystallized from light petroleum (b.p. 80–100°) in colourless prisms, m.p. 86–87°. (Found: C, 57·3; H, 4·1; S, 11·0. $C_{14}H_{12}O_5S$ requires C, 57·6; H, 4·1; S, 10·9%.)

3:5 - *Dinitro* - (3′:4′ - *methylenedioxyphenoxy)phenyl* - N - *acetyl*-L-*alanine ethyl ester*. A solution of 3:5-dinitro-*N*-acetyl-L-tyrosine ethyl ester (Chalmers, Dickson, Elks & Hems, 1949) (11·7 g.) and toluene-*p*-sulphonyl chloride (7·3 g.) in dry pyridine (10 ml.) was heated on a steam bath for 30 min. Sesamol (9·5 g., 2 mol. prop.) was added and the mixture heated in an oil bath at 130–140° for 2 hr., N_2 being bubbled through the mixture to ensure adequate mixing and to exclude O_2. When the N_2 was omitted or when higher reaction temperatures were employed, the yield of the required product was considerably reduced. The pyridine was removed under reduced pressure and the warm residue dissolved in $CHCl_3$ (100 ml.). The $CHCl_3$ solution was extracted successively with 2N-HCl, 2N-NaOH and water, after which the solvent was removed. Attempts to crystallize the residue were unsuccessful, so the crude material was dissolved in acetone (50 ml.) and passed through a column of Al_2O_3 (Hopkin & Williams 'Activated, for Chromatographic Analysis') (20 cm. × 2 cm.). Elution with the same solvent gave a clear yellow solution which, on evaporation, furnished an orange-coloured glass which could not be induced to crystallize. It was highly soluble in most organic solvents but insoluble in light petroleum. Yield: 10·5 g. (66%). A sample for analysis was obtained by distillation at 190–200° (bath)/10^{-5} mm. (Found: C, 51·4; H, 4·2; N, 9·2. $C_{20}H_{19}O_{10}N_3$ requires C, 52·0; H, 4·1; N, 9·1%.)

3:5 - *Diamino* - (3′:4′ - *methylenedioxyphenoxy)phenyl* - N - *acetyl*-L-*alanine ethyl ester*. A solution of the dinitro ester (20 g.) in methanol (150 ml.) was treated with 2% Pd on $BaSO_4$ (20 g.) and hydrogenated at 19° and 1 atm. pressure. The theoretical amount of H_2 was absorbed. The catalyst was filtered off under N_2 and the solvent removed from the colourless filtrate. The residue (16·8 g., 96%) solidified on treatment with light petroleum (b.p. 40–60°) to crystals, m.p. 58°, which rapidly discoloured on exposure to air. The base was highly soluble in methanol and ethanol but sparingly soluble in ether, light petroleum and benzene. As it could not be distilled at 210° (bath)/10^{-4} mm., it was characterized as its more stable *dihydrochloride*, which was obtained by passing dry HCl through an ethereal solution of the base containing a little methanol. The salt was washed with ether and dried, when it was obtained as a mauve-coloured solid, m.p. 191–193° (decomp.). (Found: C, 51·3; H, 6·0; N, 8·6; Cl, 12·3. $C_{20}H_{23}O_6N_3$,2HCl requires C, 50·6; H, 5·3; N, 8·9; Cl, 15·0%.)

The *diacetyl* derivative was obtained by heating the base with acetic anhydride and pyridine. It crystallized from 90% (v/v) ethanol in needles, m.p. 212·5–213°; $[\alpha]_D^{21}$ +59·9° in $CHCl_3$ (*c*, 0·22). (Found: C, 59·0; H, 5·7; N, 8·8. $C_{24}H_{27}O_8N_3$ requires C, 59·3; H, 5·6; N, 8·65%.)

3:5 - *Diiodo* - 4 - (3′:4′ - *methylenedioxyphenoxy)phenyl* - N - *acetyl*-L-*alanine ethyl ester*. The crude diamine (16·5 g.) was suspended in acetic acid (35 ml.) and dissolved by the gradual addition of H_2SO_4 (sp.gr. 1·84, 17·5 ml.) at about 20°. The solution was added dropwise over 50 min. to a stirred and cooled (−8° to −2°) solution of $NaNO_2$ (7·5 g.) in H_2SO_4 (sp.gr. 1·84, 54·5 ml.) to which acetic acid (110 ml.) had previously been added. The tetrazo solution was stirred at 0° for 40 min., then added over 10 min. to a mixture of a solution of NaI (35 g.), I_2 (29 g.) and urea (4·5 g.) in water (570 ml.) and $CHCl_3$ (200 ml.). The temperature rose to 40°. The mixture was mechanically stirred for 30 min. then left overnight. The $CHCl_3$ layer was separated, washed with 10% (w/v) $NaHSO_3$ to remove I_2, then with water. The solvent was removed and the residue, dissolved in benzene (100 ml.), passed through a column of alumina (30 cm. × 2 cm.). The product was eluted with benzene and the eluate evaporated. The residue (13 g., 51%) crystallized on treatment with ether to a solid. Pure 3:5-*diiodo*-4-(3′:4′-*methylenedioxyphenoxy)phenyl*-N-*acetyl*-L-*alanine ethyl ester* was obtained from ethanol as colourless prisms, m.p. 142–143°; $[\alpha]_D^{20}$ +8·5° in ethanol (*c*, 1·0). (Found: C, 38·5; H, 3·4; N, 2·2; I, 40·3. $C_{20}H_{19}O_6NI_2$ requires C, 38·5; H, 3·1; N, 2·2; I, 40·8%.)

3:5 - *Diiodo* - 4 - (3′:4′ - *methylenedioxyphenoxy)phenyl* - N - *acetyl*-L-*alanine*. A solution of the ethyl ester (1 g.) in ethanol (20 ml.) and N-NaOH (14 ml.) was kept at 25° for 30 min., then acidified to Congo red with HCl. The gummy material solidified on trituration with water. The solid was extracted with 0·2N-$NaHCO_3$ and the extract filtered and acidified with HCl. The *acetamido acid* (0·70 g., 73%) was washed in water and dried. It crystallized from 90% (v/v) ethanol in colourless needles, m.p. 215–216°; $[\alpha]_D^{21}$ +37·8° in 0·05N-NaOH (*c*, 1·745); $[\alpha]_D^{20}$ +10·0° in acetone (*c*, 1·45). (Found: C, 36·8; H, 2·9; N, 2·35; I, 42·4. $C_{18}H_{15}O_6NI_2$ requires C, 36·3; H, 2·5; N, 2·35; I, 42·7%.)

Attempts to remove the acetyl group by heating the acid (1·07 g.) under reflux with HCl and acetic acid gave a brown solid (0·85 g.) which dissolved readily in ethanol containing a little HCl and in alkalis, but was insoluble in most organic solvents. It charred at about 180° and evolved I_2 at about 295° without melting. The analytical figures (Found: C, 33·0; H, 3·35; I, 41·4. $C_{16}H_{13}O_5NI_2$ requires C, 34·7; H, 2·35; I, 46·0%), however, indicated that the material was not sufficiently pure for its identification to be confirmed.

Similar results were obtained when attempts were made to combine the hydrolysis and demethylation of the corresponding *N*-acetylalanine ethyl ester, by heating in N_2 with

Table 1. *R_F values for iodothyroacetic acids and iodothyronines, obtained by the ascending technique*

The compositions of the solvent systems are given in the text.

Solvent system	Substituents	R_F values: Thyroacetic acids	R_F values: Thyronines
Butanol–dioxan–ammonia	3:5:3′:5′-Tetraiodo	0·54	0·59
	3:5:3′-Triiodo	0·69	0·70
	3:5-Diiodo	0·73	0·72
*iso*Pentanol–ammonia	3:5:3′:5′-Tetraiodo	0·42	0·24
	3:5:3′-Triiodo	0·53	0·39
	3:5-Diiodo	0·64	0·50
tert.-Pentanol–ammonia	3:5:3′:5′-Tetraiodo	0·49	0·34
	3:5:3′-Triiodo	0·71	0·54
	3:5-Diiodo	0·73	0·58
n-Butanol–acetic acid–water (78:5:17)	3:5:3′:5′-Tetraiodo	0·94	0·80
	3:5:3′-Triiodo	0·94	0·80
	3:5-Diiodo	0·93	0·76

HI and acetic acid. A brown solid, decomposing at about 285°, which could not be purified, was obtained. The analytical results indicated that iodine had been lost during the process. (Found: C, 34·3; H, 3·5; N, 2·5; I, 39·2. $C_{15}H_{13}O_5NI_2$ requires C, 33·3; H, 2·4; N, 2·6; I, 47·0%.)

Chromatographic methods

Aqueous methanolic solutions containing 0·1–0·25 μg. of the three iodinated thyroacetic acids were applied to Whatman no. 1 paper, and the chromatograms developed by the ascending technique with solvents previously found capable of separating triiodothyronine from thyroxine, i.e. the upper phases of butanol–dioxan–2N-NH_3 soln. (4:1:5) (Gross, Leblond, Franklin & Quastel, 1950), *tert.*-pentanol–2N-NH_3 soln. (1:1) (Gleason, 1955), and 'amyl alcohol' (B.D.H. 'purified for milk testing')–6N-NH_3 soln. (1:1). In this laboratory the last-named mixture has proved more satisfactory for the separation of the iodothyronines than the purified *iso*pentanol–6N-NH_3 soln. mixture of Roche, Deltour, Michel & Velez (1953).

The iodothyroacetic acids were located on the chromatograms by means of the ceric sulphate–arsenious acid reagent of Bowden, Maclagan & Wilkinson (1955). As expected, all three substances proved highly sensitive to this reagent, the minimum quantities detectable being: diiodo, 0·1 μg.; triiodo, 0·025 μg.; tetraiodo, 0·01 μg.

The R_F values obtained are listed in Table 1. It will be noticed that in butanol–dioxan–ammonia the thyroacetic acids have R_F values similar to those of the corresponding thyronines, but in the other ammoniacal solvents tetraiodothyroacetic acid is indistinguishable from triiodothyronine.

SUMMARY

1. The acetic acid analogues of thyroxine and triiodothyronine have been synthesized from *p*-hydroxyphenylacetic acid.

2. A synthetic route to 3:5-diiodo-4-(3′:4′-dihydroxyphenoxy)phenyl-L-alanine and its 5′-iodo derivative, possible products of the hydrolytic deiodination of triiodothyronine and thyroxine respectively, has been explored.

3. The R_F values of the iodothyroacetic acids in a number of different solvent systems have been compared with those of the corresponding iodothyronines.

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