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O-Phosphoric Acid Esters of 3,5-Diiodotyrosine and Thyroxine

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As part of a study of the mechanism of action of the thyroid hormones and their congeners, *O*-phosphoric acid esters of these compounds were needed in order to test the possibility that phosphorylation of the phenolic hydroxyl group occurs during metabolic alterations. Tyrosine-*O*-phosphoric acid had been prepared by heating tyrosine with polyphosphoric acid,[1] and this procedure appeared to offer the simplest approach to analogous esters of iodinated derivatives. However, preliminary experiments showed that this method is not applicable to iodinated aromatic amino acids, since elementary iodine was liberated under the conditions required for the reactions. Phosphorus oxychloride has frequently been used to synthesize phenolic monoesters of phosphoric acid at low temperatures, but the use of this reagent with phenolic amino acids requires that the amino and carboxyl groups be blocked. The carboxyl group is usually esterified with methanol or ethanol for this purpose, and the intermediary esters can later be hydrolysed by dilute alkali under mild conditions; this protective pathway seemed applicable to the sensitive iodinated tyrosines and thyronines. Protection of the amino group of such compounds must be achieved in such a manner that the removal of the blocking groups will leave the phosphate ester linkage intact. This rules out standard acylation of the amino groups, and even *N*-carbobenzoxylation because the aromatic

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iodine atoms do not stand up under the reductive removal of the carbobenzoxy group. Since the *N*-trifluoroacetyl group can be cleaved with 0·2 N sodium hydroxide solution, and the *N*-trifluoroacetyl derivatives of 3,5-diiodotyrosine and thyroxine were known,[2] *N*-trifluoroacetylation appeared to offer the best promise for our purpose.

Taurog, Abraham and Chaikoff[2] had worked with DL-thyroxine, but the adaptation of their directions to the L-isomer offered considerable difficulty, chiefly because of the low solubility of L-thyroxine methyl ester. This was overcome by conducting the *N*-trifluoroacetylation of this compound in Diglyme solution. In the case of diiodotyrosine ethyl ester, *N*-trifluoroacetylation proceeded best without any solvent.

The phosphorylation of *N*-trifluoroacetyl-3,5-diiodotyrosine ethyl ester with phosphorus oxychloride in pyridine gave 69 per cent of the *O*-phosphoric acid. Removal of the protecting groups by alkaline hydrolysis gave the water-soluble 3,5-diiodotyrosine-*O*-phosphoric acid which was purified via its insoluble lead salt. The phosphorylation of *N*-trifluoroacetyl-L-thyroxine methyl ester furnished a 51 per cent yield of product; alkaline hydrolysis of the blocking groups gave the water-insoluble L-thyroxine-*O*-phosphoric acid which precipitated as a hydrate on addition of hydrochloric acid.

Experiments directed toward an analogous synthesis of 3,3′,5-triiodothyronine-*O*-phosphoric acid were handicapped by low yields. The yield of methyl ester was 25 per cent, and *N*-trifluoroacetylation gave only 30 per cent of the amide. The yield of an impure product from the phosphorylation reaction was so low that this sequence had to be abandoned.

L-Thyroxine-*O*-phosphoric acid was administered intraperitoneally to three rats in daily doses of 28·2, 33·6 and 52·1 micrograms per 100-gram rat, respectively. After 6 days of injections, the metabolisms of the rats increased by 39·9, 53·6 and 52·8 per cent. The injections were discontinued after 10 days. One rat died 2 days later, and a second rat died 4 days after the injections were discontinued. The third rat survived and the metabolism returned to the base line. The biological testing will be continued when larger amounts of material become available.

Experimental*

N-*Trifluoroacetyl-3,5-diiodo-*L*-tyrosine ethyl ester.* A mixture of 3,5-diiodo-L-tyrosine ethyl ester[3] (4·6 g, 0·01 mole) and redistilled trifluoroacetic anhydride (3·5 g, 0·017 mole) was ground thoroughly in a mortar and then allowed to stand for 30 min. The pasty mass was then ground well with 100 ml of water and the mixture was filtered. The undissolved material was dissolved in a small volume of ethyl acetate and precipitated with hexane, yielding 2·3 g (54 per cent based on converted starting material), m.p. 167–170°(d.); reported,[2] m.p. 172–173°.

From the acid filtrate, 1·1 g of unreacted starting material was recovered by treatment with sodium bicarbonate solution.

N-*Trifluoroacetyl-3,5-diiodo-*L*-tyrosine ethyl ester* O-*phosphoric acid.* A solution of *N*-trifluoroacetyl-3,5-diiodo-L-tyrosine ethyl ester (2·8 g, 0·0061 mole) in dry pyridine (5 ml) was cooled to −10°, and a solution of phosphorus oxychloride (0·86 g, 0·0057 mole) in dry pyridine (8 ml) was added dropwise with stirring over a period of 15 min. The solution was kept at −10° for 2 h and at 25° for 1 h. It was then carefully decomposed with 30 g of ice, followed by 100 ml of water. The pyridine was neutralized with dilute sulphuric acid to pH 7, and a yellow gummy precipitate was filtered off. An excess of sulphuric acid was then added, and the white precipitate was filtered, washed, and dried, giving 2·2 g (69 per cent) of material, m.p. 177–180°.

An analytical sample was obtained by three recrystallizations from ethanol followed by one from acetone. The product crystallized with one molecule of acetone and melted at 185–187°(d.).

Anal. Calcd. for $C_{13}H_{13}F_3I_2NO_7P.C_3H_6O$: C, 27·65; H, 2·76; I, 36·26. Found: C, 27·53; H, 2·79; I, 36·51.

*3,5-Diiodo-*L*-tyrosine* O-*phosphoric acid.* A solution of *N*-trifluoroacetyl 3,5-diiodo-L-tyrosine ethyl ester *O*-phosphoric acid (0·90 g) in 1N sodium hydroxide (11·2 ml) was shaken at 25° for 2 h, and then acidified with 30 per cent acetic acid. A 10 per cent lead acetate solution was added till precipitation of the insoluble lead salt was complete. The precipitate was filtered off, washed with water, and suspended in 20 ml of water. The suspension

* All melting points are corrected. Microanalyses by Mrs. Dolores Ellis.

was stirred while hydrogen sulphide was passed in for 10 h. Even so, the low solubility of the lead salt seemed to prevent complete decomposition. The lead sulphide was filtered off and the clear solution was concentrated in vacuum. When the volume was reduced to about 2 ml, all the product quickly crystallized. The colourless crystals were filtered, washed with a small amount of cold water, and dried, yielding 0·18 g (25 per cent), m.p. 216°(d.). Addition of ethanol to the filtrate gave a negligible amount of a flocculent white precipitate.

Anal. Calcd. for $C_9H_{10}I_2NO_6P$: C, 21·07; H, 1·97. Calcd. for hemihydrate: C, 20·71; H, 2·12. Found: C, 20·92; H, 2·30.

*3,3′,5-Triiodo-*DL*-thyronine methyl ester.* A stream of dry HCl was passed into a suspension of 12 g of 3,3′,5-triiodo-DL-thyronine* in 50 ml of methanol. The solid went into solution quickly, and the solution was then saturated with hydrogen chloride. After cooling to 25° HCl was again passed in to saturation. The solution was then concentrated to 4 ml under reduced pressure, 15 ml of ether was added, and the precipitate was filtered and dried; yield, 0·52 g, m.p. 209–210°(d.). The material was dissolved in 50 per cent ethanol (10 ml) and the solution was adjusted to pH 7 with 1 N sodium hydroxide. After the precipitate had settled out it was filtered, dried, and recrystallized from benzene, giving 0·30 g (25 per cent) of colourless crystals, m.p. 178·5–179°(d.).

Anal. Calcd. for $C_{16}H_{14}I_3NO_4$: C, 28·90; H, 2·12; I, 57·25. Found: C, 28·95; H, 2·35; I, 56·78.

N-*Trifluoroacetyl 3,3′,5-triiodo-*DL*-thyronine methyl ester.* A mixture of 3,3′,5-triiodo-DL-thyronine methyl ester (0·28 g, 0·00042 mole) and trifluoroacetic anhydride (0·15 g, 0·00072 mole) was ground thoroughly in a mortar, allowed to stand for 15 min, and then ground well with water (10 ml), followed by 5 per cent sodium bicarbonate solution (10 ml). The solid material was filtered off, dried, and extracted with dry ether (10 ml), leaving 0·05 g of unreacted ester. Addition of hexane (20 ml) to the extract precipitated another 0·08 g of unreacted ester. The solution was then heated on a steam bath until all the ether had boiled off. The hexane solution on cooling deposited 0·05 g

* We are grateful to Smith Kline and French Laboratories for a gift of 10 grams of this material.

of colourless crystals (30 per cent based on unrecovered starting material), m.p. 165–169°(d.). Two recrystallizations from methylene chloride–hexane raised the melting point to 173–174·5°(d.).

Anal. Calcd. for $C_{18}H_{13}F_3I_3NO_5.H_2O$: C, 28·41; H, 1·72; I, 50·03. Found: C, 28·32; H, 1·90; I, 49·69.

N-*Trifluoroacetyl* L-*thyroxine methyl ester.* To a solution of L-thyroxine methyl ester[4] (1·8 g, 0·0023 mole) in Diglyme (45 ml) (dried and distilled over lithium aluminium hydride) was added all at once a solution of trifluoroacetic anhydride (0·75 g, 0·0036 mole) in Diglyme (5 ml). After standing for 2 h, 30 ml of water was added and when the gummy yellow precipitate had settled, the solution was decanted, and the remainder of the solute precipitated by addition of 100 ml of water. The precipitate was filtered and dried; it was then extracted with methylene chloride (20 ml), leaving 0·3 g of unreacted starting material. The extract was then treated with hexane (40 ml), precipitating another 0·3 g of unreacted starting ester. The solution was concentrated to 30 ml under reduced pressure and the light yellow precipitate was filtered and dried. It weighed 0·6 g (45 per cent based on starting material not recovered), and melted at 165–168°.

Anal. Calcd. for $C_{18}H_{12}F_3I_4NO_5$: C, 24·38, H, 1·36. Found: C, 24·48; H, 1·51.

N-*Trifluoroacetyl* L-*thyroxine methyl ester* O-*phosphoric acid.* A solution of *N*-trifluoroacetyl L-thyroxine methyl ester (0·32 g, 0·00036 mole) in dry pyridine (0·6 ml) was cooled to −10°, and a solution of phosphorus oxychloride (0·10 g, 0·00067 mole) in dry pyridine (1 ml) was added dropwise. The mixture was kept for 1 h at −10° and for 30 min at 25°, and poured into 5 per cent hydrochloric acid (30 ml). A granular yellow solid precipitated. It is important to acidify the solution this way instead of adding the acid to the solution, to prevent precipitation of insoluble pyridinium salts which cannot be filtered. The precipitate was filtered, dried, and dissolved in acetone (3 ml). By fractional precipitation with benzene a middle fraction of 0·18 g (51 per cent), m.p. 194–196° (d.) was obtained. The material crystallized with one mole of acetone.

Anal. Calcd. for $C_{18}H_{13}F_3I_4NO_8P.C_3H_6O$: C, 24·60; H, 1·87; I, 50·02. Found: C, 24·17; H, 1·64; I, 50·47.

L-*Thyroxine* O-*phosphoric acid.* A solution of *N*-trifluoroacetyl

L-thyroxine methyl ester *O*-phosphoric acid (0·030 g) in 1N sodium hydroxide (1 ml) was kept at 25° for 8 h and then acidified with dilute hydrochloric acid. The colourless precipitate was filtered, washed, and dried. It weighed 0·015 g (56 per cent) m.p. 212–214°(d.).

Anal. Calcd. for $C_{15}H_{12}I_4NO_7P$: C, 21·03; H, 1·41. Calcd. for the monohydrate: C, 20·60; H, 1·61. Found: C, 20·79; H, 1·68.

Summary. A synthesis of 3,5-diiodo-L-tyrosine *O*-phosphoric acid and L-thyroxine *O*-phosphoric acid has been performed. The latter ester significantly increased the metabolism of rats.

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