Summary

The preparation of *dl*-β-cyclohexylalanine and several derivatives is described.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLUMBIA UNIVERSITY]

An Analog of Thyroxine

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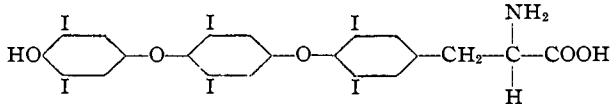
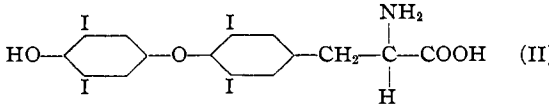
There have been numerous studies on the chemical constitution of thyroxine in relation to its physiological activity. A full discussion of this subject is given by Harington[1] in his monograph on thyroxine. He observes that the presence of certain chemical features in the molecule seems to be responsible for its specific physiological activity, namely, its iodine content, the special orientation of two of the iodine atoms in the ortho position to a phenolic group, the presence of the diphenyl ether linkage, and the α-amino acid residue.

It therefore seemed of some interest to see whether the following compound (I) might possess thyroxine activity.



(I)

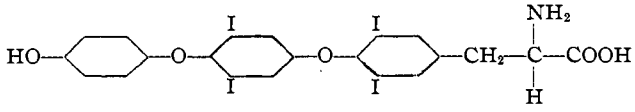
Comparing this with thyroxine (II) the chemical similarities are shown.



(II)

Physiologically, however, the three-ringed amino acid showed no significant activity.

One of the intermediate products in the synthesis is the following



(III)

Because of the parallel relationship between this and our final product (I) on the one hand, and 3,5-diiodothyronine and thyroxine on the other, it was thought worth while to test this physiologically. It showed no more activity than that displayed by the fully iodinated compound (I).

(1) Harington, "The Thyroid Gland," Oxford University Press, London, 1933, Chapter VII.

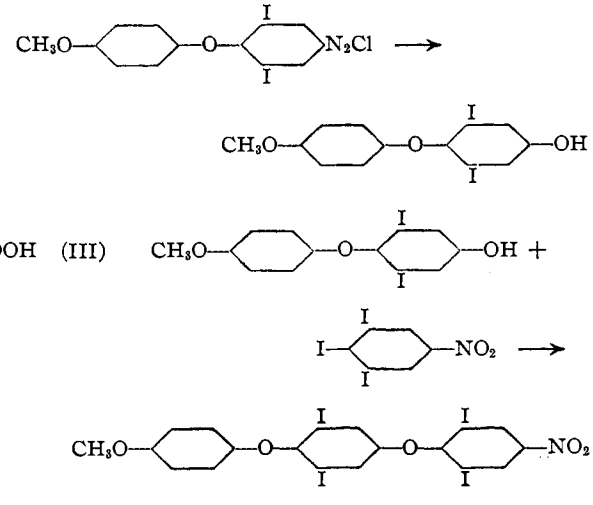
Experimental

Bio-assay.—Since the preliminary estimations, which were done by comparing the weight curves of test animals with thyroxine treated and normal controls, showed so little activity, the more complicated and tedious assay by basal metabolic rates was not undertaken.

Guinea pigs of about 300 g. weight were used. All animals were fed the same adequate diet *ad lib.* The results are shown in Chart I. Each curve represents a group of six animals. Injections and weighings were done daily. The controls received 1 cc. of warm water; the thyroxine and new substances were injected as sodium salts in 1 cc. of warm water. The dosage of thyroxine was 0.05 mg. per animal per day, that of the new compounds 2 mg. per animal per day. The injections were given intraperitoneally.

Since the animals tested with the new substances, although not gaining weight quite as rapidly as the untreated controls, showed no effects comparable to the thyroxine controls, and since the molecular dosage of new substances was about thirty times that of thyroxine, it seems safe to conclude that there is very little, if any, thyroid activity in either of the new compounds.

The synthetic procedure was similar to that followed by Harington and Barger[2] for thyroxine. The only new steps introduced were the following



A 20% solution of 3,5-diiodo-4-(4'-methoxyphenoxy)-benzene diazonium chloride in glacial acetic acid was pre-

(2) Harington and Barger, *Biochem. J.*, **21**, 169 (1927).

pared according to Harington and Barger.[2] This was added to a hot (100–110°), vigorously stirred 50% (by weight) sulfuric acid solution and both temperature and stirring maintained for twenty to thirty minutes, when the β-naphthol test for diazonium salts became negative. The orange mixture turned yellow-gray, and a dark brown sediment separated out. After cooling and standing overnight the solid was filtered off and extracted with 0.5 *N* sodium hydroxide. On addition of an equal volume of 4 *N* sodium hydroxide to the resulting solution the crystalline sodium salt of the phenol separated out. This was centrifuged, washed with 2 *N* sodium hydroxide, dissolved in water, and the solution was made acid to congo with hydrochloric acid. The precipitate was recrystallized from aqueous alcohol or acetic acid. The phenol crystallized as slightly brown, short needles or rods, m. p. 160–163°, yield 48%.

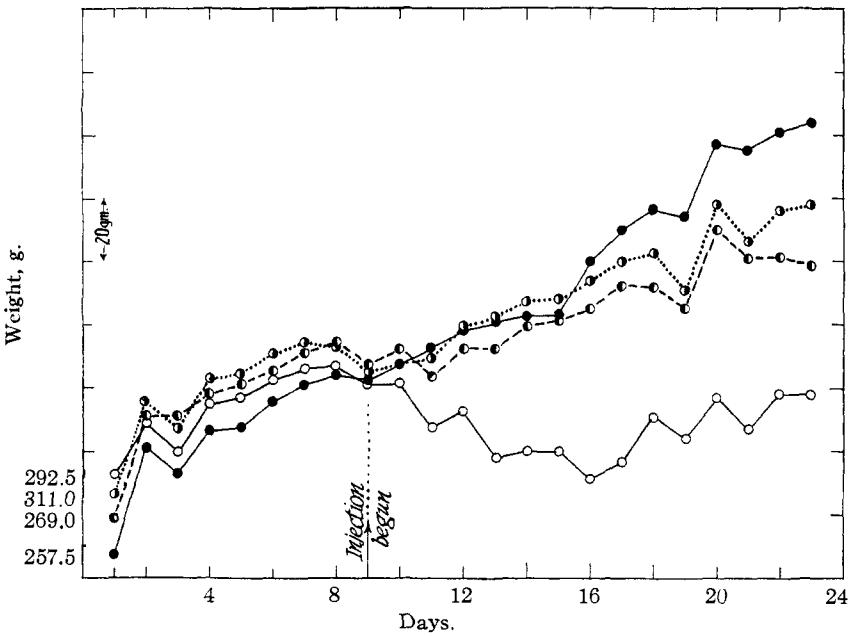


Fig. 1.—●—●, Control; ○—○, thyroxine; ◑···◑, thyroxine *p*-hydroxy phenyl ether; ◐– –◐, thyroxine 4-hydroxy-3,5-diiodo phenyl ether.

Anal. Calcd. for $C_{13}H_{10}O_3I_2$: C, 33.3; H, 2.12; I, 54.0. Found: C, 33.2; H, 2.18; I, 53.7.

3,5 - Diiodo - 4 - [3',5' - diiodo - 4' - (4" - methoxyphenoxy)-phenoxy]-nitrobenzene.—A mixture of 26 g. of the phenol, 8.8 g. of anhydrous potassium carbonate, 31.2 g. of 3,4,5-triiodonitrobenzene, and 125 cc. of dry methyl ethyl ketone was refluxed for fifteen hours on the steam-bath. The hot mixture of salt and yellow product which precipitated out of solution was filtered and washed with the ketone and then with water; 60 g. of almost pure product was left on the filter. The mother liquors on cooling yielded 12 more grams, and still more on dilution with water. The product crystallized in long yellow needles or rods, m. p. 190–192°; yield 87%.

Anal. Calcd. for $C_{19}H_{11}O_5NI_4$: C, 27.0; H, 1.30; I, 60.4; OCH_3, 3.7. Found: C, 27.0; H, 1.35; I, 60.3; OCH_3, 3.6.

3,5 - Diiodo - 4 - [3',5' - diiodo - 4' - (4" - methoxyphenoxy)-phenoxy]-aminobenzene.—Dry hydrogen chloride was bubbled through a solution of 150 cc. of hot acetic acid containing 15 g. of the trinitro compound and 17 g. of crystallized stannous chloride. The reaction flask was kept hot on the steam-bath. After a while the crystalline stannichloride of the amine began to separate out (one hour). The mixture was cooled and allowed to stand in the icebox overnight. The crystalline precipitate was filtered off and washed with a little cold acetic acid. Then in small portions it was shaken vigorously in a separatory funnel with saturated potassium hydroxide and a large volume of peroxide-free ether containing a little stannous chloride. If no stannous chloride is added, colored products are formed on shaking with alkali, which apparently can be reduced by addition of stannous chloride. The amine is sparingly soluble in ether and large quantities were used, each portion of ether being siphoned off when saturated. The ether solution was filtered and concentrated, whereupon the amine came out as white needles. When hydrochloric acid was passed into the ethereal solution of amine, the hydrochloride separated out as prisms. These were unstable and on being dried lost hydrochloric acid to give the free amine, m. p. 185–187°; yield 87%.

Anal. Calcd. for $C_{19}H_{13}O_3NI_4$: C, 28.1; H, 1.6; I, 62.6. Found: C, 28.0; H, 1.8; I, 62.4.

3,5 - Diiodo - 4 - [3',5' - diiodo - 4' - (4" - methoxyphenoxy)-phenoxy]-benzonitrile.—Diazotization of the amine was done most conveniently in small lots. To each solution of 1.8 g. (4 *M*) of ethyl nitrite in 40 cc. of glacial acetic acid at room temperature was added slowly and with occasional shaking a 5-g. portion of dry amine. The amine dissolved slowly as the reaction proceeded. The resulting clear orange solutions were combined and added dropwise, at room temperature, and with continuous stirring, to a cuprous cyanide solution prepared by adding, for each 20 g. of amine, 160 g. of potassium cyanide in 280 cc. of water to 120 g. of cupric sulfate in 520 cc. of water. After completion of the reaction as indicated by absence of positive naphthol test for diazonium salt, the mixture was filtered. The light brown solid was dried over phosphorus pentoxide *in vacuo*, and then extracted with benzene solution. The dark brown benzene solution was passed through an aluminum oxide column, losing most of its color and coming out pale yellow. This solution was evaporated to dryness *in vacuo* and redissolved in acetone. On diluting the acetone solution with one-fourth its volume of water and allowing it to stand overnight, a flocculent yellow precipitate came out. This was filtered off and the practically colorless filtrate on further dilution yielded the nitrile as long colorless needles. On purification by crystallization

from acetone–water these melted at 225–226°; yield 64%.

Anal. Calcd. for $C_{20}H_{11}O_3NI_4$: C, 29.2; H, 1.34; N, 1.7; I, 61.8. Found: C, 29.1; H, 1.34; N, 1.8; I, 61.8.

3,5 - Diiodo - 4 - [3′,5′ - diiodo - 4′ - (4″ - methoxyphenoxy)-phenoxy]-benzaldehyde.—To a solution of 6.9 g. of freshly fused, anhydrous stannous chloride in 35 cc. of anhydrous ether saturated with dry hydrogen chloride gas was added 5 g. of nitrile dissolved in 40 cc. of anhydrous chloroform. There was a slight evolution of heat and hydrogen chloride gas, after which the container was tightly stoppered and allowed to stand at room temperature. A yellow, flocculent precipitate soon began to form. After twenty-four hours the heavy yellow precipitate was filtered off, washed with a little cold, dry ether, and suspended in dilute hydrochloric acid. The mixture was brought to boiling and kept there for five minutes. The precipitate became white and granular. It was filtered off and recrystallized from a water–acetone mixture, coming out as clusters of lancets, m. p. 196–198°; yield 70%.

Anal. Calcd. for $C_{20}H_{12}O_4I_4$: C, 29.1; H, 1.54; I, 61.7. Found: C, 28.9; H, 1.50; I, 61.3.

Azlactone of β-3,5-Diiodo-4-[3′,5′-diiodo-4′-(4″-methoxyphenoxy) - phenoxy] - phenyl - α - acetaminoacrylic Acid.—An intimate mixture of 2 g. of aldehyde, 0.28 g. of acetylglycine, 1.2 g. of freshly fused anhydrous sodium acetate, and 4 cc. of acetic anhydride was refluxed on the oil-bath for one hour. After cooling the yellow solid was filtered off, washed with cold acetic anhydride, then with water till the washings came through neutral, dried and recrystallized from acetic anhydride; glistening golden plates, m. p. 264–265°; yield 80%.

Anal. Calcd. for $C_{24}H_{15}O_5NI_4$: C, 31.8; H, 1.76; I, 56.1. Found: C, 31.8; H, 1.65; I, 55.9.

Thyroxine-*p*-hydroxyphenyl Ether.—To a suspension of 2 g. of red phosphorus in a mixture of equal parts (by volume) of acetic anhydride and 57% hydriodic acid was added 2.8 g. of azlactone and the mixture refluxed on the oil-bath for one and one-quarter hours. The phosphorus was removed by filtering the hot mixture through asbestos. Occasionally the hydroiodide of the amino acid separated out of the hot solution during the course of the reaction, but was brought into solution previous to removal of the phosphorus by addition of more hot dilute acetic acid. After removal of the acetic acid and hydriodic acid as completely as possible by repeated addition of small amounts of water and evaporation *in vacuo*, the insoluble light brown solid was filtered off, washed with a little dilute alcohol, suspended in a large volume of hot alcohol, and brought into solution by the addition of a few drops of *N* sodium hydroxide. On acidification with acetic acid and cooling the amino acid came out as clusters of long colorless needles; 1.75 mg. was obtained. This product was very difficult to purify. There was some loss of iodine during the reduction and hydrolysis, the analysis at this point always being 1 to 2% too low. On repeated recrystallization as described above, the product gradually approached analytical purity, but the yield suffered greatly; 200 mg. of pure material was prepared for analysis and bioassay, m. p. 267–268° with decomposition.

Anal. Calcd. for $C_{21}H_{15}O_5NI_4$: C, 29.0; H, 1.7; N, 1.61; I, 58.46. Found: C, 28.9; H, 1.9; N, 1.67; I, 58.30.

Thyroxine-4-hydroxy-3,5-diiodophenyl Ether.—Because of the very slight solubility of the tetraiodo compound in aqueous ammonia, the iodination was carried out in a methyl alcohol–aqueous ammonia mixture.

To a solution of 869 mg. of tetraiodo compound in a mixture of 140 cc. of methyl alcohol and 200 cc. of 25% aqueous ammonia was added dropwise 8 cc. of 0.5 *N* iodine in 0.1 *M* potassium iodide. If more than the calculated amount of iodine was used, there was an increase in destruction, probably due to oxidation. The iodine was taken up rapidly. After addition of the iodine the solution was put in the icebox for several hours. Most of the ammonia was then removed by distillation *in vacuo* at room temperature, and the solution was acidified with acetic acid. The white precipitate obtained weighed 1.1 g. A portion of this, 415 mg., was extracted with hot 95% ethyl alcohol, the solution filtered and diluted to 70% with water. A crop of fine white needles came out. This was filtered off, redissolved in 100 cc. of hot alcohol and diluted with 30 cc. of water; 130 mg. of fine white needles was thus obtained. This compound was rather unstable, coloration and loss of iodine occurring on heating both in solution and in the dry state. It could only be thoroughly dried by heating in high vacuum, and even when kept dry and unexposed to light, there was brown coloration and loss of iodine. Because of these properties it was difficult to purify. Decomposition starts at 210° without melting.

Anal. Calcd. for $C_{21}H_{13}O_5NI_6$: C, 22.48; H, 1.16; N, 1.25; I, 67.9. Found: C, 22.26; H, 1.23; N, 1.34; I, 67.5.

Summary

The synthesis of the 3,5-diiodo-4-hydroxyphenyl ether of thyroxine is described. The product is physiologically inactive.

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