lected solvent gave no residue. Development with another 10-ml. portion of morpholine caused the yellow band to appear fainter and 78 mg. of residue was obtained on distilling the collected solvent. Two crystallizations from ethanol yielded 10 mg. of erysonine of m. p. 238–239° (original erysonine observed simultaneously melted also at 238–239°) and $(\alpha)_D$ +280 (*c* 7.336 mg. in 2.033 ml. 0.5% aqueous hydrochloric acid).

A solution was made by dissolving 120 mg. of erysonine (same material as dissolved in morpholine) in 50 ml. of a 50–50 mixture of methanol and chloroform.

This solution was passed into a 30 × 1.9 cm. column of aluminum oxide Merck (according to Brockmann) without suction. No solvent passed through. The ends of the tube were stoppered and the tube was observed under ultraviolet light. There was a 1 mm. band on the very top of the green fluorescence which corresponds to a slight amount of decomposition products and this material was not recovered. There were six similar striations placed at approximately 4, 6, 10, 14.5, 20, and 25 cm. from the top which had a pale light yellow fluorescence under ultraviolet light. There was a thin brighter line which seemed to be near the edge of the advancing solvent line. On developing with 50 ml. of the same mixed solvents, the striations moved down about 1 cm. each and the collected solvent gave only 1 mg. of residue.

The adsorbent was separated into two portions under the ultraviolet light. All the fluorescent portions were combined and the non-fluorescent portions were combined. The top thin layer containing the decomposed material was discarded. Both portions were eluted by continuous hot extraction for eight hours with the same mixed solvent. After distillation of the solvent from the fluorescent portions, 30 mg. of residue was obtained which yielded 12 mg. of erysonine on crystallization from ethanol; m. p. 235–236°, $(\alpha)_D$ + 282 (*c* 9.685 mg. in 2.023 ml. of 0.5% aqueous hydrochloric acid). The solvent from the non-fluorescent portions yielded 65 mg. of residue after distillation. Recrystallization of this material from ethanol yielded 30 mg. of erysonine, m. p. 236–237, $(\alpha)_D$ + 279 (*c* 19.307 mg. in 2.033 ml. of 0.5% aqueous hydrochloric acid).

These two chromatographic analytical experiments did not yield erysonine of altered melting point or specific rotation when compared to the original crystallized material.

Acknowledgment.—We wish to express our gratitude to Mr. B. A. Krukoff of the New York Botanical Garden for obtaining the plant materials, for determination of specimens, and for his advice on botanical matters. We are indebted to the many people who, aiding in the collection of plant material, have helped to make these studies possible. Acknowledgment is made to Dr. Klaus Unna of the Merck Institute for Therapeutic Research for his coöperation on the pharmacological assays. The technical assistance of Mr. W. B. Wright and the microanalyses by Messrs. D. F. Hayman, W. Reiss, and H. S. Clark are gratefully acknowledged.

Summary

The liberated alkaloidal fractions obtained from the seeds of nine species of *Erythrina* have been examined. Erysodine and erysopine were isolated in many cases. One new liberated alkaloid named erysonine was isolated from the seeds of one species.

Erysonine has the empirical formula $C_{17}H_{19}NO_3$. Certain preliminary facts about its constitution have been described. Erysonine possesses a curare-like action in frogs when administered by intralymphatic injection. The threshold dose is 100 mg./kg.

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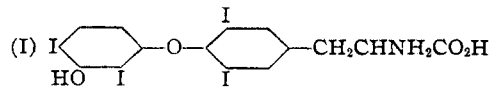
The Synthesis of *dl*-3,5-Diiodo-4-(2′,4′-diiodo-3′-hydroxyphenoxy)-phenylalanine, a Physiologically Inactive Isomer of Thyroxine

BY CARL NIEMANN AND C. E. REDEMANN

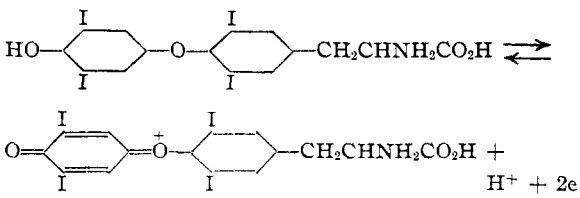
Although the structure of the physiologically active *l*-thyroxine has been known for a number of years, there is little information as to how this amino acid performs its characteristic physiological function. Harington[1] and, more recently, Bovarnick, Bloch and Foster[2] have set forth the obligatory structural requirements, as far as they are known, for the development of thyroxine-like activity but offer no suggestion as to why these requirements are necessary. In order to extend our knowledge along these lines, it seemed of interest to us to synthesize an isomer of thyroxine in which the hydroxyl group in the second ring was shifted from position 4′, as in thyroxine, to position 3′. This compound, *dl*-3,5-diiodo-4-(2′,4′-diiodo-3′-hydroxyphenoxy)-phenylalanine (I), was prepared and when tested on rats was found

(1) C. R. Harington, *Fortschritte Chem. organ. Naturstoffe*, **2**, 103 (1939).

(2) M. Bovarnick, K. Bloch and G. L. Foster, THIS JOURNAL, **61**, 2472 (1939).



to be inactive, even in doses of 500 mg. per kg. of body weight.[3] This observation taken in conjunction with those of Harington[1] and others[4] suggested the provisional working hypothesis that thyroxine-like activity, at least in the case of thyroxine, is dependent upon the establishment of the equilibrium



It is therefore predicted that those structures which do not permit the formation of a quinoid form will be inactive and that quantitative differences in the activity of those compounds which can form such structures are due to the influence of nuclear substituents on the oxidation–reduction potential of the system as a whole.[5,6] Experiments designed to test this hypothesis are now in progress.

In conclusion we wish to point out that the synthetic procedure reported in this communication has also been employed for the synthesis of *dl*-thyroxine. In general we have found that the yields in the case of thyroxine are comparable to those recorded here for its isomer and that the procedure possesses greater applicability than does the original procedure of Harington and Barger[7] from which it was derived. The authors wish to express their indebtedness to Messrs. H. Lanz, Jr., and G. A. Swinehart for the microanalyses reported in this paper.

(3) We are indebted to Professor P. Phillips and Mr. P. D. Boyer, Department of Biochemistry, University of Wisconsin, for this information. A complete report of their work will appear elsewhere.

(4) (a) K. Schuegraf, *Helv. Chim. Acta*, **12**, 405 (1929); (b) E. Abderhalden and E. Wertheimer, *Z. ges. exptl. Med.*, **63**, 557 (1928); (c) K. H. Slotta and K. H. Soremba, *Ber.*, **69**, 566 (1936).

(5) It is possible that the amino acid side chain is not only involved in the quantitative effect but is also necessary for an independent process in which the amino acid is incorporated into a protein or peptide.

(6) Bovarnick, Bloch and Foster[2] claim that 3,5-diiodo-[3′,5′-diiodo-4′-(3″,5″-diiodo-4″-hydroxyphenoxy)-phenoxy]-phenylalanine is physiologically inactive. Since these authors did not administer the compound at levels higher than approximately 6 mg./kg., and since a slight activity apparently was observed, we do not believe that it can be concluded, at least at present, that this compound is completely devoid of physiological activity as would be demanded for an incompatibility with the proposed hypothesis.

(7) C. R. Harington and G. Barger, *Biochem. J.*, **21**, 169 (1927).

Experimental

2,6-Diiodo-4-nitroaniline.—The directions of Sandin, Drake and Leger[8] did not give complete satisfaction until they had been modified in the following respects. In place of heating on a boiling water-bath the reaction mixture was refluxed for two hours in an oil-bath. After cooling the reaction mixture to room temperature the solid was recovered, pressed into a firm cake, and sucked as dry as possible. The cake was then transformed into a smooth paste with 600 ml. of hot water, a little sodium bisulfite added to remove excess iodine, and the suspension filtered. The residue was dried at 70–75° for sixteen hours and gave 340 g. (86%) of yellow flakes, m. p. 245°. Stout yellow prisms, m. p. 249–250°, were obtained by recrystallizing the crude product from nitrobenzene.

3,4,5-Triiodonitrobenzene.—The following method based upon the procedure of Hodgson and Walker[9] for diazotizing weakly basic amines was found to give results more satisfactory than any published method.

To a well-stirred suspension of 100 g. of powdered 2,6-diiodo-4-nitroaniline in 600 ml. of acetic acid, cooled to 15°, was added slowly a filtered solution of 25 g. of sodium nitrite in 140 ml. of concd. sulfuric acid. The temperature was kept below 25° during the addition of the nitrosyl sulfuric acid solution and stirring was continued until all of the diiodonitroaniline had dissolved. The resulting deep yellow solution was poured into 2 liters of ice water and urea added to destroy the excess nitrous acid. The solution was filtered and to the vigorously stirred filtrate was added slowly a solution of 60 g. of potassium iodide in 300 ml. of water. The reaction mixture was heated to 85–90° in a water-bath, cooled to room temperature, the excess iodine removed with sodium bisulfite, the light buff-colored precipitate of triiodonitrobenzene recovered, washed, and dried to constant weight; crude yield 128 g. (99%), m. p. 157–160°. A single recrystallization from cellosolve gave dark yellow crystals, m. p. 164–166° (recovery 88%). Purification of the crude triiodonitrobenzene by distillation gave a product, m. p. 164–166°, which was somewhat lighter in color.

Resorcinol Monomethyl Ether.—This compound was prepared by the method of Dey, Rao and Seshadri[10] in 76% yield, b. p. 118–120° (8–9 mm.).

3,5-Diiodo-4-(3′-methoxyphenoxy)-nitrobenzene.—It was found that the procedure of Harington and Barger[7] was improved by changing to a solvent of slightly higher boiling point and by using a smaller excess of the phenol. Their ratio of 2:1 for phenol to nitro compound gave an excessive amount of tarry by-products; a reduction of the ratio to 1.4:1 greatly improved this condition.

A mixture of 88 g. of 3,4,5-triiodonitrobenzene, 35 g. of resorcinol monomethyl ether, 45 g. of freshly dehydrated anhydrous potassium carbonate and 180 ml. of freshly distilled 2-pentanone was refluxed in an oil-bath for six hours. Water was added to dissolve the salts and the 2-pentanone and other volatile products were removed by steam distillation. When 1.5 liters of distillate had been

(8) R. B. Sandin, W. V. Drake and F. Leger, "Organic Syntheses," Vol. XII, 1932, p. 28.

(9) H. H. Hodgson and J. Walker, *J. Chem. Soc.*, 1620 (1933).

(10) B. B. Dey, R. H. R. Rao and T. R. S. Seshadri, *J. Indian Chem. Soc.*, **12**, 140 (1935).

collected, the dark brown residue in the distillation flask crystallized to form a loose crumbly mass. After cooling the solid was recovered, washed well with water and cold methanol, and finally dried. Upon recrystallization from methyl ethyl ketone 67 g. (76%) of light yellow rhombic plates, m. p. 140–141°, was obtained. The substance can be recrystallized from methyl ethyl ketone, glacial acetic acid or cellosolve.

Anal. Calcd. for $C_{13}H_9O_4NI_2$ (497.1): C, 31.4; H, 1.8; N, 2.8. Found: C, 31.5; H, 2.0; N, 2.9.

3,5-Diiodo-4-(3′-methoxyphenoxy)-aniline.—To a hot solution of 45 g. of 3,5-diiodo-4-(3′-methoxyphenoxy)-nitrobenzene in 180 ml. of acetic acid was added in small portions, with some cooling, 68 g. of stannous chloride dihydrate. The solution was then saturated with dry hydrogen chloride while the temperature was maintained at 95–100°. When the solution became nearly saturated with hydrogen chloride the white stannic chloride double salt of the amine separated in abundant quantity. After saturation with hydrogen chloride the mixture was allowed to cool to room temperature and the double salt of the amine was recovered. This was sucked as dry as possible and was then transferred to a flask surrounded by an ice-bath and containing 300 ml. of ether. The ethereal suspension was vigorously stirred while 100 ml. of 50% sodium hydroxide solution was added dropwise. The ethereal extract was decanted from the pasty residue and the latter was extracted with two additional 200 ml. portions of ether. The combined ether extract was decolorized by adding 0.5 g. of stannous chloride, the solution dried over anhydrous sodium sulfate, and the ether removed by distillation first at atmospheric pressure and finally under reduced pressure. A cream colored crystalline residue of nearly pure amine weighing 38 g. (90%) remained in the flask. For analysis a sample of the amine was recrystallized from a mixture of benzene and petroleum ether giving short blunt colorless prisms, m. p. 135–136°. The amine is readily soluble in most organic solvents with the exception of petroleum ether.

Anal. Calcd. for $C_{13}H_{11}O_2NI_2$ (467.1): C, 33.4; H, 2.3; N, 3.0. Found: C, 33.6; H, 2.5; N, 3.3.

The hydrochloride of this amine could not be prepared in a pure state. From dry ether it precipitated as a sticky gum and from dry benzene or warm concd. hydrochloric acid it separated in the form of fine needles which lose hydrogen chloride upon drying. Various preparations melted at temperatures varying from 86–123° with the evolution of gas and analyses were always between those calculated for the amine and the amine hydrochloride.

3,5-Diiodo-4-(3′-methoxyphenoxy)-aniline Picrate.—To a moderately concentrated warm alcoholic solution of the amine was added a saturated alcoholic solution of picric acid and after standing for several days one large yellow roset of crystals (no special form could be distinguished) of the amine picrate separated, m. p. 156–157°. The picrate cannot be recrystallized without decomposition into amine and picric acid.

Anal. Calcd. for $C_{19}H_{14}O_9N_4I_2$ (696.2): C, 32.8; H, 2.0; N, 8.0. Found: C, 32.5; H, 1.9; N, 8.0.

3,5-Diiodo-4-(3′-methoxyphenoxy)-acetanilide.—This compound was prepared by the action of acetic anhydride upon the corresponding amine. It crystallized only after long standing in rosets of colorless needles from 70% ethanol, m. p. 176–177°.

Anal. Calcd. for $C_{15}H_{13}O_3NI_2$ (509.1): C, 35.4; H, 2.6; N, 2.8. Found: C, 35.6; H, 3.0; N, 2.6.

3,5-Diiodo-4-(3′-methoxyphenoxy)-benzonitrile.—The method of Harington and Barger[7] could not be applied directly since the dry hydrochloride of the amine could not be prepared. Attempts to diazotize the amine when dissolved in glacial acetic acid led to the formation of a deep orange insoluble condensation product, probably the diazoamino compound, which was not broken up by excess nitrous acid. The following procedure was finally developed.

Twenty grams of 3,5-diiodo-4-(3′-methoxyphenoxy)-aniline was dissolved in 200 ml. of warm glacial acetic acid, the solution rapidly cooled to 15°, and 25 ml. of glacial acetic acid containing 1.5 g. of dry hydrogen chloride added. Diazotization was achieved by slowly introducing 7 ml. of freshly distilled *n*-butyl nitrite to the above solution while maintaining the temperature below 20°. After standing for thirty minutes the diazonium solution was added dropwise to a vigorously stirred potassium cuprocyanide solution prepared from 114 g. of potassium cyanide, 100 g. of cupric sulfate pentahydrate, and 700 ml. of water and maintained below 10° during the addition of the diazonium solution. The reaction mixture was then stirred for one hour at room temperature and finally warmed to 80°. After the solution was cooled to 20° the solid was collected and dried first at 20° for sixteen hours and then at 60–70°, under reduced pressure, for twenty-four hours. The solid was thoroughly extracted with two 300-ml. portions of boiling benzene and the dark brown benzene extract passed through a Tswett column charged with activated alumina.[2] After removing the benzene from the yellow filtrate the residue weighed 13.7 g. Upon recrystallization from a mixture of ethyl methyl ketone and ethyl alcohol a total of 12.5 g. of crystalline material was obtained. This was a solvate, m. p. 149–151°; yield 60%. For analysis a small sample was distilled at 0.01 mm. pressure, m. p. 156–157°.

Anal. Calcd. for $C_{14}H_9O_2NI_2$ (477.1): C, 35.2; H, 1.9; N, 2.9. Found: C, 35.2; H, 2.0; N, 2.9.

3,5-Diiodo-4-(3′-hydroxyphenoxy)-benzoic Acid.—A specimen of the 3,5-diiodo-4-(3′-methoxyphenoxy)-benzonitrile was hydrolyzed with a 1:1 mixture of acetic acid and hydriodic acid (d. 1.7) for one-half hour. The hydrolyzate was then diluted with cold water, the precipitate recovered, extracted with dilute ammonium hydroxide, the extract filtered, and the filtrate acidified with 6 *N* hydrochloric acid. The precipitated acid was collected and recrystallized from 30% aqueous ethanol, fine white needles, m. p. 203° with decomposition.

Anal. Calcd. for $C_{13}H_8O_4I_2$ (482.1): C, 32.3; H, 1.7. Found: C, 32.4; H, 1.8.

3,5-Diiodo-4-(3′-methoxyphenoxy)-benzaldehyde.—Forty grams of anhydrous stannous chloride was suspended in 220 ml. of anhydrous ether and dry hydrogen chloride passed into the suspension at 0° until all of the solid had dissolved and only a single liquid phase remained. To this solution was added a solution of 16 g. of 3,5-diiodo-4-(3′-

methoxyphenoxy)-benzonitrile dissolved in 70 ml. of dry chloroform. The flask was then stoppered and allowed to stand at room temperature. After about two hours a second heavy yellow liquid phase separated. After standing for twenty-four hours no solid phase had formed; therefore, the ether and excess hydrogen chloride was removed on a water-bath and when the volume had been reduced to about one-third of the initial amount the yellow stannic chloride double salt of the aldimine hydrochloride separated rapidly. After the solution had cooled the solid was collected and hydrolyzed by boiling with 6 *N* hydrochloric acid. The aldimine double salt hydrolyzed rapidly leaving a yellow crystalline solid which was washed with water and dried; weight 12 g. (75%). For analysis a sample was recrystallized from 70% aqueous acetic acid giving stout colorless needles, m. p. 145–146°.

Anal. Calcd. for $C_{14}H_{10}O_3I_2$ (480.1): C, 35.0; H, 2.1. Found: C, 35.1; H, 2.3.

The above aldehyde was converted into the corresponding 2,4-dinitrophenylhydrazone, microscopic orange-red needles from glacial acetic acid, m. p. 276–277°, in the usual manner.

Anal. Calcd. for $C_{20}H_{14}O_6N_4I_2$ (660.1): C, 36.4; H, 2.1; N, 8.5. Found: C, 36.4; H, 2.2; N, 8.4.

4(3′,5′-Diiodo-4′-(3″-methoxyphenoxy)-benzal)-2-phenyloxazolone-5.—An intimate mixture of 9 g. of 3,5-diiodo-4-(3′-methoxyphenoxy)-benzaldehyde, 3.4 g. of hippuric acid, 9 g. of anhydrous sodium acetate and 40 ml. of acetic anhydride was heated in a boiling water-bath for one hour. The dark colored reaction mixture was poured, with stirring, into 800 ml. of ice water and allowed to stand until the acetic anhydride had hydrolyzed. The orange-yellow solid was collected, washed with water, and dried in vacuo. The 11.5 g. of azlactone thus obtained is not very pure but is satisfactory for subsequent operations. For analysis a sample of the crude azlactone was twice extracted with boiling ethanol, then crystallized from cellosolve, washed with ethanol and dried. This product sintered at 163° and melted at 166–168°.

Anal. Calcd. for $C_{23}H_{15}O_4NI_2$ (623.1): C, 44.3; H, 2.4; N, 2.3. Found: C, 43.9; H, 2.5; N, 2.7.

α-Benzoylamino-β-(3,5-diiodo-4-(3′-methoxyphenoxy)-phenyl)-acrylic Acid.—To 500 ml. of a boiling 2% solution of sodium hydroxide in 50% ethanol was added 10 g. of the above azlactone. After boiling the solution for six minutes the pale yellow solution was filtered and the filtrate acidified with 6 *N* hydrochloric acid. The acid began to separate immediately and after cooling, at 0°, for several hours the solid was collected and dried giving 7.0 g. of a cream-colored product. For analysis the acid was dissolved in 60% aqueous ethanol by adding the requisite quantity of sodium hydroxide and then reprecipitated by adding 6 *N* hydrochloric acid. The final product was composed of fine colorless needles, m. p. 212–213°.

Anal. Calcd. for $C_{23}H_{17}O_5NI_2$ (641.1): C, 43.1; H, 2.7; N, 2.2. Found: C, 43.2; H, 2.7; N, 2.1.

***dl*-α-Amino-β-(3,5-diiodo-4-(3′-hydroxyphenoxy)-phenyl)-propionic Acid.**—A mixture of 25 ml. of acetic anhydride, 25 ml. of hydriodic acid (d. 1.7), 3 g. of red phosphorus and 5.0 g. of the benzoylaminocinnamic acid was refluxed for one and one-quarter hours. The hot solution was filtered through a sintered glass filter and the filtrate evaporated to dryness *in vacuo*. The residue was boiled with 2 *N* hydrochloric acid for a few minutes, filtered and the hot filtrate neutralized with 15 *N* ammonium hydroxide whereupon the amino acid began to crystallize immediately in the form of small colorless platelets. The amino acid was collected, washed with alcohol and dried, weight 1.62 g. (39%). After recrystallization the amino acid melted at 229–231°.

Anal. Calcd. for $C_{15}H_{13}O_4NI_2 \cdot H_2O$ (543.1): C, 33.2; H, 2.8; N, 2.6. Found: C, 33.1; H, 2.9; N, 2.8.

***dl*-α-Amino-β-(3,5-diiodo-4-(2′,4′-diiodo-3′-hydroxyphenoxy)-phenyl)-propionic Acid.**—A solution of 844 mg. of 3,5-diiodo-4-(3′-hydroxyphenoxy)-phenylalanine in 10 ml. of 15 *N* ammonium hydroxide and 10 ml. of water was cooled in an ice–salt-bath and to this solution was added dropwise with effective stirring the theoretical amount of 1 *M* iodine in potassium iodide. Throughout the addition the temperature was maintained below 5°. When all of the potassium triiodide solution had been added, the solution was allowed to stand in the ice-bath for one hour. The solution was then diluted with 40 ml. of water and sulfur dioxide was passed in for five minutes. The resulting red-purple solution was slowly neutralized with 6 *N* hydrochloric acid. When the reaction mixture was about one-half neutralized a dark purple gummy precipitate separated. At this point an equal volume of ethanol was added which caused the precipitate to dissolve and a light purple solution remained. When neutrality was reached a nearly colorless flocculent precipitate separated and within a few minutes this precipitate became crystalline and completely colorless. After standing overnight the precipitate was collected, washed successively with water and ethanol, and dried *in vacuo;* weight 1.04 g. (87%), m. p. 202°.

Anal. Calcd. for $C_{15}H_{11}O_4NI_4$ (776.9): C, 23.2; H, 1.4; N, 1.8; I, 65.4. Found: C, 23.2; H, 1.6; N, 1.6; I, 65.0.

Summary

The synthesis of *dl*-3,5-diiodo-4-(2′,4′-diiodo-3′-hydroxyphenoxy)-phenylalanine, an isomer of thyroxine, is described. This compound was found to be inactive when tested on rats at levels up to 500 mg. per kg. of body weight.

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