a few days' standing, and this peroxide formation led to erroneous results, especially in the measurement of densities and indices of refraction. The physical constants were determined with the same precision as reported in our previous paper.[3]

Acetylenes.—The monoalkylacetylenes were prepared in the usual manner from alkyl bromides and sodium acetylide in liquid ammonia.[8] The dialkylacetylenes were prepared from alkyl bromides, sodium acetylide and sodamide in liquid ammonia.[9] For the introduction of methyl and ethyl groups the alkyl sulfates were used in preference to the bromides. In the preparation of unsymmetrical dialkylacetylenes the larger group was introduced first.[5] The use of this "one-step" method was satisfactory for all of the dialkylacetylenes reported except 2-octyne. In this case it is preferable to isolate the 1-heptyne and then methylate it, since otherwise purification is difficult owing to the close boiling points of amyl bromide and 2-octyne. The acetylenic carbinols were prepared by the method of Campbell, Campbell and Eby.[10]

Catalytic Hydrogenations.—The procedure previously described[3] was used to prepare the *cis*-olefins, and care was taken to keep the reaction temperature below 60°. For the 1-alkenes it was found better to stop the hydrogenation shortly before the calculated amount of hydrogen had been absorbed, since it is easier to free the olefin from acetylene than from saturated hydrocarbon.

(8) Hennion, *Proc. Ind. Acad. Sci.*, **47**, 116 (1938).
(9) Bried and Hennion, THIS JOURNAL, **59**, 1310 (1937).
(10) Campbell, Campbell and Eby, *ibid.*, **60**, 2882 (1938).

The saturated hydrocarbons were prepared in 0.1 mole quantities; the products from several runs were then combined, washed several times with concentrated sulfuric acid to remove any unsaturates, then with water, dried and fractionated.

Sodium–Liquid Ammonia Reductions.—These were carried out as described earlier.[3]

Summary

1. The *cis* forms of 2-hexene and 2-octene have been prepared by catalytic hydrogenation of dialkylacetylenes, and the *trans* forms by sodium–liquid ammonia reduction.

2. The sodium–liquid ammonia method has been applied to the preparation of 1-alkenes and found to yield pure substances.

3. Two acetylenic carbinols have been converted to the olefinic carbinols by both methods of reduction.

4. The physical constants of the isomeric straight-chain hexenes and octenes have been determined, and the relationship between the physical constants and the position of the double bonds has been discussed.

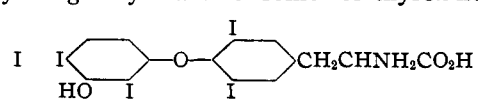
NOTRE DAME, INDIANA RECEIVED JULY 31, 1941

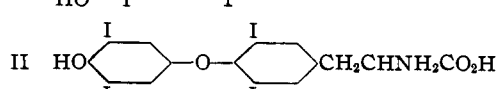
[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 848]

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

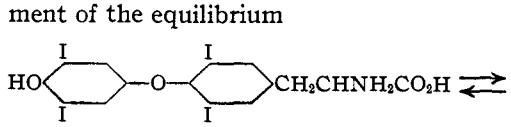
BY CARL NIEMANN AND JAMES F. MEAD

In a previous communication from this Laboratory[1] the synthesis of *dl*-3,5-diiodo-4-(2′,4′-diiodo-3′-hydroxyphenoxy)-phenylalanine (I), a physiologically inactive isomer of thyroxine (II),

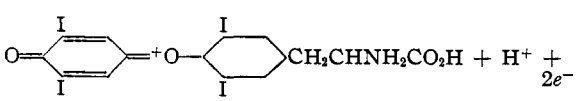




was described, and it was suggested that thyroxine-like activity is dependent upon the establishment of the equilibrium

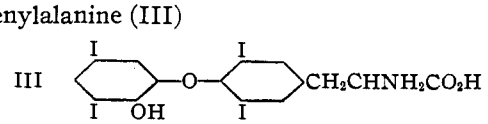


(1) C. Niemann and C. E. Redemann, THIS JOURNAL, **63**, 1549 (1941).



It was therefore predicted that those isomers of thyroxine which could not be converted into a quinoid structure like that above would be inactive and that quantitative differences in the activity of those compounds which could form such structures would be due to the influence of nuclear substituents on the oxidation–reduction potential of the system as a whole.

As a test of this hypothesis we have synthesized *dl*-3,5-diiodo-4-(3′,5′-diiodo-2′-hydroxyphenoxy)-phenylalanine (III)



and have found that this isomer of thyroxine, in

accordance with the prediction, is physiologically active, the activity being approximately one twenty-fifth of that of *dl*-thyroxine.[2] We therefore conclude that the synthesis of this physiologically active isomer of thyroxine, which is the first physiologically active isomer of thyroxine ever synthesized,[1,3] has resulted in further support for the proposed hypothesis relating chemical structure and thyroxine-like activity.

Experimental

The experimental procedure employed for the synthesis of *dl*-3,5-diiodo-4(3',5'-diiodo-2'-hydroxyphenoxy)-phenylalanine was patterned after the original thyroxine synthesis of Harington and Barger[4] and is given in detail only in those instances where experimental difficulties may be encountered.

3,5-Diiodo-4-(2'-methoxyphenoxy)-nitrobenzene (IV).—A mixture of 250 g. of triiodonitrobenzene,[1] 90 g. of guaiacol, 155 g. of freshly dehydrated anhydrous potassium carbonate and 650 ml. of freshly distilled 2-pentanone was refluxed for six hours. Water was added and the 2-pentanone and excess guaiacol were removed by steam distillation. The water was decanted and, after cooling, the tarry mass was treated with 500 ml. of methanol, which dissolved out most of the tar and caused the residue to solidify; 250 g. of this residue was recrystallized three times from 2-butanone to give 80 g. of light yellow crystals, m. p. 148–150°.

Anal. Calcd. for $C_{13}H_9O_4NI_2$ (497.0): C, 31.4; H, 1.8; N, 2.8. Found: C, 31.2; H, 2.0; N, 2.9.

3,5-Diiodo-4-(2'-methoxyphenoxy)-aniline Hydrochloride (V).—To a hot solution of 75 g. of IV in 375 ml. of acetic acid was added, in small portions, 115 g. of powdered stannous chloride dihydrate and the stannic chloride double salt of the amine isolated as previously described.[1,4] The stannic chloride double salt was ground in a mortar with 150 ml. of warm 50% sodium hydroxide solution, the suspension diluted with 100 ml. of ice water, and exhaustively extracted with ether. Dry hydrogen chloride was passed into the dried ethereal extract precipitating the amine hydrochloride which was then collected with the aid of acetone. The product (60 g.) melted at 237° after preliminary sintering.

Anal. Calcd. for $C_{13}H_{12}O_2NI_2Cl$ (503.5): C, 31.0; H, 2.4; N, 3.0. Found: C, 30.9; H, 2.5; N, 3.0.

The free base was liberated from the above hydrochloride by shaking an ethereal suspension of the latter compound with 2 *N* aqueous sodium hydroxide. The base, recovered from the ethereal solution, was acetylated with acetic anhydride and after several recrystallizations from ethanol, 3,5-diiodo-4-(2'-methoxyphenoxy)-acetanilide, m. p. 225–227°, was obtained as colorless platelets.

Anal. Calcd. for $C_{15}H_{13}NO_3I_2$ (509.1): C, 35.4; H, 2.6; N, 2.8. Found: C, 35.7; H, 2.6; N, 2.9.

(2) We are indebted to Professor P. Phillips, Department of Biochemistry, University of Wisconsin, for this information. A complete report of the physiological work will appear elsewhere.

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

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

3,5-Diiodo-4-(2'-methoxyphenoxy)-benzonitrile (VI).—Butyl nitrite (10 g.) was added to a well-stirred suspension of 40 g. of V in 400 ml. of acetic acid containing 5% of water. Upon warming to 50° a clear orange solution was obtained, which was added, with stirring, to a solution prepared by adding 245 g. of potassium cyanide in 400 ml. of water to 215 g. of cupric sulfate pentahydrate in 800 ml. of water. The mixture was warmed to 80°, cooled, filtered and the solid first dehydrated and then extracted with hot benzene.[1] The dark brown solution was decolorized by passing it through a column of activated alumina, and from the filtrate 31 g. of light yellow crystals was obtained. This product was then distilled at 0.2 mm. (bath temp. 225°) to give 25 g. of nitrile. The distilled nitrile was recrystallized from ethanol to give light yellow prisms of VI, m. p. 135–137°.

Anal. Calcd. for $C_{14}H_9NO_3I_2$ (477.1): C, 35.3; H, 1.9; N, 2.9. Found: C, 35.2; H, 2.0; N, 2.8.

A specimen of VI was treated with acetic acid and hydriodic acid as previously described[1] to give 3,5-diiodo-4-(2'-hydroxyphenoxy)-benzoic acid, colorless needles, m. p. 253–254°, with preliminary sintering.

Anal. Calcd. for $C_{13}H_8O_4I_2$ (482.0): C, 32.4; H, 1.7. Found: C, 32.7; H, 1.8.

3,5-Diiodo-4-(2'-methoxyphenoxy)-benzaldehyde (VII).—VI (14.5 g.) was treated with anhydrous stannous chloride as previously described[1] to give 8 g. (55%) of VII, m. p. 137–140°, after recrystallization from acetic acid. The *p*-nitrophenylhydrazone of VII was prepared in the usual manner[1] to give yellow needles, m. p. 249–250°.

Anal. Calcd. for $C_{20}H_{15}N_3O_4I_2$ (615.1): C, 39.0; H, 2.5; N, 6.8. Found: C, 39.3; H, 2.6; N, 6.5.

4-(3',5'-Diiodo-4'-(2''-methoxyphenoxy)-benzal)-2-phenyloxazalone-5 (VIII).—A nearly quantitative yield of crude azlactone (VIII) was obtained from the above aldehyde (VII) by following the procedure previously described.[1] The crude azlactone was recrystallized from cellosolve to give yellow needles, m. p. 198–200°.

Anal. Calcd. for $C_{23}H_{15}O_4NI_2$ (623.2): C, 44.3; H, 2.4; N, 2.3. Found: C, 44.5; H, 2.6; N, 2.1.

***dl*-α-Amino-β-(3,5-diiodo-4-(2'-hydroxyphenoxy)-phenyl)-propionic Acid (IX).**—A mixture of 20 ml. of acetic anhydride, 20 ml. of hydriodic acid (d. 1.7), 3 g. of red phosphorus and 3 g. of azlactone (VIII) was refluxed for four hours. The hot solution was filtered and the filtrate evaporated to dryness *in vacuo*. The residue was boiled for one minute with 2 *N* hydrochloric acid, cooled, filtered, the filtrate heated just to boiling, exactly neutralized with dilute aqueous ammonia and immediately filtered, The filtrate was allowed to stand overnight, the precipitate collected, washed with water and ethanol and again reprecipitated as described above. The crude amino acid was then dissolved in hot 80% ethanol with the aid of dilute aqueous sodium hydroxide, the solution filtered and the filtrate adjusted to *p*H 6.0 with dilute aqueous acetic acid. After standing for some time IX crystallized as clusters of colorless needles, m. p. 240°, with decomposition. Approximately 0.3 g. of recrystallized IX was obtained from 3 g. of VIII.

Anal. Calcd. for $C_{15}H_{13}NO_4I_2$ (525.1): C, 34.3; H,

2.5; N, 2.7; I, 48.3. Found: C, 34.5; H, 2.8; N, 2.5; I, 48.6.

dl-α-Amino-β-(3,5-diiodo-4-(3′,5′-diiodo-2′-hydroxyphenoxy)-phenyl)-propionic Acid (X).—Iodine (0.28 g.) dissolved in 1 *M* aqueous potassium iodide was added, dropwise, to a chilled solution containing 0.277 g. of IX in 10 ml. of 7 *N* ammonium hydroxide, and the reaction mixture allowed to stand at 0° for one-half hour. After the addition of a small amount of sodium bisulfite solution to the reaction mixture, it was adjusted to *p*H 4 with dilute hydrochloric acid. The solid that had precipitated was collected and washed with water and ethanol. The crude amino acid was then recrystallized by dissolving it in 80% ethanol containing the requisite quantity of sodium hydroxide and suddenly acidifying the solution with dilute acetic acid to give 0.33 g. of X, pale pink clusters of needles, m. p. 218–219°, with decomposition.

Anal. Calcd. for $C_{15}H_{11}O_4NI_4$ (776.9); C, 23.2; H, 1.4; N, 1.8; I, 65.3. Found: C, 23.5; H, 1.7; N, 1.9; I, 65.4.

Summary

The synthesis of *dl*-3,5-diiodo-4-(3′,5′-diiodo-2′-hydroxyphenoxy)-phenylalanine, an isomer of thyroxine, is described. This compound is physiologically active, the activity being approximately one twenty-fifth of that of *dl*-thyroxine. This finding is in accordance with an earlier prediction and is taken as evidence in favor of a proposed hypothesis relating chemical structure and thyroxine-like activity.

PASADENA, CALIFORNIA RECEIVED AUGUST 4, 1941

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNITED GAS IMPROVEMENT CO.]

An Anomalous Bromination Reaction. The Analytical Bromination of Styrene and Indene by the Kaufmann Method

BY C. W. JORDAN

Kaufmann[1,2] developed a method for the determination of bromine numbers of various unsaturated organic compounds using a solution of bromine in methanol saturated with sodium bromide. With pure, water-free reagents a 0.1 *N* solution retained a constant titer over a much longer period of time than any other bromine solution tested. He observed that when bromine was added to methanol saturated with sodium bromide the solution was bright yellow in contrast with the red solutions formed by the same concentration of bromine in carbon tetrachloride, chloroform, glacial acetic acid, etc. The vapor pressure of the bromine appeared to be greatly lowered since the solution had very little odor. This was an important factor in preventing loss of bromine. The physical changes noted led to the conclusion that the halogen no longer existed in a free state but was loosely attached to the sodium bromide forming the tribromide, $NaBr_3$, or in some manner was combined with the methanol, or both.

By analogy with the known compound, sodium triiodide, the constitution was believed to be either $NaBr\langle^{Br}_{Br}$ or $Na\langle^{Br}_{Br}Br$.

Kaufmann concluded that this solution was the outstanding titration liquid for use in bromometry in that it was odorless, permanent and easy to pipet in contrast with bromine solutions in carbon tetrachloride, glacial acetic acid, etc. It reacted far more energetically than iodine and completely saturated the double bond without the formation of substitution products.

Analytical Procedure.—The standard bromine solution was prepared by saturating Kahlbaum methanol with pure anhydrous sodium bromide (100 parts of methanol dissolve 12–15 parts of sodium bromide) and then adding bromine in the amount required to form 0.1 *N* solution. The exact normality was established by adding an excess of 0.2 *N* potassium iodide and titrating the liberated iodine with 0.1 *N* sodium thiosulfate. The quantity of sample recommended for test varied between 0.1 g. and 1.0 g. depending upon the magnitude of the bromine number.

The substance under test was weighed from a dropping bottle and put in a dry 300-ml. Erlenmeyer flask containing 15 ml. of chloroform. Chloroform was recommended for use when the substance under test was not freely miscible with the methanol–bromine solution. An excess of bromine solution was then added at room temperature and the mixture allowed to react, usually for two hours. The unreacted bromine was titrated iodimetrically and the bromine or iodine number calculated in the usual manner. The index of reliability of the method was determined by titrating a number of pure unsaturated compounds.

Anomalous Reactions Using Kaufmann Bromine Solution.—Kaufmann verified the method solely by determining the total amount of bromine consumed. Since the values obtained

(1) H. P. Kaufmann and E. Hansen-Schmidt, *Archiv. der Pharmazie*, **263**, 32 (1925).

(2) H. P. Kaufmann, *Zeit. f. Untersuchung der Lebensmittel*, **51**, 3–14 (1926).