The Conversion of DL-3:5-Diiodo-4-hydroxyphenyllactic Acid into an Analogue of Thyroxine

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In extending the work of Ludwig & von Mutzenbecher (1939) on the iodination of proteins under mildly alkaline conditions, leading to the production of thyroxine within the protein molecule, von Mutzenbecher (1939) illustrated the probable mechanism involved by showing that 3:5-diiodotyrosine, on standing at comparable pH, was converted, in small yield, into thyroxine. The subsequent literature dealing with the conversion of free diiodotyrosine into thyroxine has been cited by Harington & Pitt Rivers (1945; see also Reineke & Turner, 1946), who investigated the conditions of the reaction in some detail and postulated an oxidative mechanism, involving iodine, which they suggest may be of biological significance.

There appears to be no report indicating whether or not derivatives of diiodotyrosine have been found to undergo spontaneous transformation to the corresponding analogues of thyroxine under similar conditions. Of special interest in this connexion is 3:5-diiodo-4-hydroxyphenyllactic acid, which Foster & Gutman (1930) isolated from the urine of rabbits in receipt of large doses of diiodotyrosine. Its identity was ascertained by analysis and by a consideration of its chemical behaviour, but was not confirmed by synthesis.

The present communication is a brief report of the synthesis of DL-3:5-diiodo-4-hydroxyphenyllactic acid, and of the results of preliminary experiments designed to effect its conversion into DL-3:5-diiodo-4-(3':5'-diiodo-4'-hydroxy)phenoxyphenyllactic acid.

EXPERIMENTAL

Synthesis of DL-3:5-diiodo-4-hydroxyphenyllactic acid

The 4-hydroxyphenyllactic acid required for iodination was obtained from the azlactone of 4-acetoxy- α -acetamidocinnamic acid by two-stage hydrolysis to the corresponding pyruvic acid, followed by reduction.

Azlactone of 4-acetoxy- α -acetamidocinnamic acid. Acetyl-glycine (5.85 g.), 4-hydroxybenzaldehyde (6.1 g.), acetic anhydride (13.4) and fused sodium acetate (3 g.) were refluxed for 1 hr., cooled, and placed overnight at 0°. The mass of yellow-brown crystals was broken up with a rod after the addition of 12 ml. of water. The solid was filtered, washed with ice water and dried *in vacuo* over KOH. Yield (crude): 8.7 g. (85%). The crude material melted at 128°.

4-Acetoxy- α -acetamidocinnamic acid. The crude azlactone (5 g.) was boiled with water (200 ml.) for 10 min. and the hot mixture filtered with gentle suction. The filtrate on

cooling deposited yellow crystals which were filtered, washed with water and dried at 110°. Yield: 2.6 g. (48%); m.p. 224°. A specimen recrystallized from water (Norite) gave: N, 5.30%. (Calc. for $C_{13}H_{13}O_5N$, 5.32%.) The hydrolysis of the azlactone with water gave better yields than the acetone-water mixture recommended for the corresponding derivative of benzaldehyde (*Organic Syntheses*, 2, 519).

4-Hydroxyphenylpyruvic acid. The acetamido acid (2.63 g.) was refluxed for 4 hr. in an all-glass apparatus with 2N-HCl (35 ml.) and placed overnight at 0°. The crystals of the keto acid were filtered and dried *in vacuo*. The crude material (1.0 g. (56%); m.p. 210°; Neubauer (1909), has recorded m.p. 220° for purified material) was reduced soon after preparation as some specimens became pasty on standing.

DL-4-Hydroxyphenyllactic acid. The crude keto acid (2 g.) was shaken for 10 min. at room temperature with sodium amalgam (1.25%; 130 g.) and water (25 ml.). The separated and acidified (2N-HCl) solution was extracted thrice with an equal volume of ether. The dried ethereal extract deposited an oil on evaporation, which rapidly solidified to a mass of colourless needles. Yield: 1.3 g. (64%). Recrystallized from ether-light petroleum, the substance melted at 120°, resolidified and then gave a m.p. of 135°. (Erlenmeyer & Lipp (1883) record m.p. 115–122° and 139–140° respectively.) On checking, at a later date, this specimen was found to have m.p. 137–139°, without prior change. Found (after drying at 80°): C, 59.4; H, 5.27. (Calc. for $C_9H_{10}O_4$: C, 59.3; H, 5.49%.)

DL-3:5-Diiodo-4-hydroxyphenyllactic acid. The method of iodination used, adapted from that described by Zeynek (1929) for the preparation of 3:5-diiodotyrosine, has been found suitable in this laboratory for the production of a range of diiodophenols. Iodination with iodine monochloride, as utilized by Block & Powell (1943) for the formation of diiodotyrosine, did not yield a satisfactory product.

A solution of 4-hydroxyphenyllactic acid (1.22 g.) in 2N-H₂SO₄ (13.7 ml.) and 10% (w/v) KI (29.4 ml.) was stirred rapidly at 0° with an electric stirrer. Iodic acid (10% (w/v); 15.6 ml.) was then added dropwise from a funnel, followed by glycine (2 g.) in water (30 ml.) to act as a buffer. 2N-NaOH (16 ml.) was then added over 20 min. to adjust the pH to 8 (approx.). Stirring was continued at 0° for 1 hr. and the mixture then allowed to come to room temperature, when it was acidified with 2N-HCl (6 ml.) and extracted thrice with 50 ml. of ether. The ethereal solution was washed with the minimum amount of aqueous NaHSO₄ to remove excess iodine and then with water. The oily residue, after removal of the ether from the still moist solution, was extracted successively with 40, 20 and 10 ml. of boiling water. On cooling in ice, 1.25 g. (44%) of colourless needles were deposited. The crystals lost water at 75° and then melted at 164° (Foster & Gutman (1930) report 73–75° and 158–159° respectively for their only

partly racemic material). The acid is readily soluble in organic solvents and sparingly soluble in cold water. When boiled in water at pH 7.5 the solution darkened appreciably in colour; after 2 hr. the pH had dropped to 5.5 and it was not found possible to isolate any of the original material. The apparent destruction of the lactic acid under these conditions is in accord with the findings for diiodotyrosine (Harington & Pitt Rivers, 1945; Dickinson & Trikojus, 1947). A specimen was purified for analysis by recrystallization from water and drying at 100° *in vacuo*. Found: C, 24.6; H, 2.08; I, 59.1. (Calc. for $C_9H_9O_5I_2$: C, 24.9; H, 1.84; I, 58.5%.)

Experiments on the transformation of 3:5-diiodo-4-hydroxyphenyllactic acid

The iodinated lactic acid was incubated at pH (initial) 8.8–9.8 and at temperatures of 37 or 55°, with and without the passage of oxygen. The best yield of transformation product was obtained by the following procedure; a careful comparison with other sets of conditions was not, however, carried out.

The lactic acid (1.0 g.) was dissolved in NaOH (0.1N; 23 ml.) and 1N-NaOH added to bring the pH to 8.8. The solution was incubated at 37° for 7 days, a stream of oxygen being provided by passage through a sintered-glass gas distributor. On the 2nd and 4th days *n*-butanol (5 ml.) was added. At the conclusion of the incubation period, sufficient 10N-NaOH was added to the reaction product to make the aqueous phase normal with respect to this reagent. Further butanol was added to equalize the volumes. The butanolic extract was shaken with an equal volume of 2N-NaOH and then evaporated to dryness under diminished pressure. The residue was acidified with 2N-acetic acid and the precipitate purified by dissolving in hot 50% (v/v) acetic acid (1 ml.), filtering from a small residue of amorphous material and then suddenly chilling the hot solution. The yield at this stage was 28 mg. while 350 mg. of pure starting material was recovered. On recrystallization of a portion from dilute acetic acid, an equivalent total recovery of 18 mg. was obtained, the nett yield (about 3%) of this analytically pure material being thus comparable to those obtained by Harington & Pitt Rivers (1945) and Reineke & Turner (1946) in the conversion of diiodotyrosine into thyroxine. Found (after drying at 100°): I, 65.2%. (Calc. for $C_{15}H_{10}O_5I_4$: I, 65.35%.)

It crystallized in colourless prisms, m.p. 176–177° (with prior softening), and gave an intense reddish purple colour when treated in *n*-butanol with aqueous alkali and diazotized *N'*-diethylsulphanilamide, a reagent developed in this laboratory for the estimation of thyroxine (Winikoff & Trikojus, 1948). Under appropriate conditions 3:5-diiodo-4-hydroxyphenyllactic acid and 3:5-diiodotyrosine do not give a positive reaction, so that the reagent provides a convenient test in transformations and other studies in this series. The m.p. (173°) recorded for the ketonic acid analogous with thyroxine (Canzanelli, Guild & Harington, 1935) is close to that of our compound, while the iodine value would not differentiate between the two formulae. Although a pure specimen of the keto acid was not available, an examination of the infra-red absorption of our compound was undertaken. A solution in morpholine, 5 mg./ml., was examined in the region 5.50–6.50 μ and only one band, at 6.3 μ , characteristic of the carboxylate ion, was found to be present. By contrast, two strong absorption bands, at 5.89 and 6.3 μ , were observed for α -ketoglutaric acid under similar conditions. The first of these is exactly in the correct position for a ketonic carbonyl group. We have, therefore, assumed that the transformation product of 3:5-diiodo-4-hydroxyphenyllactic acid obtained in these experiments is the lactic acid analogue of thyroxine.

SUMMARY

DL-3:5-Diiodo-4-hydroxyphenyllactic acid has been synthesized and its behaviour studied under conditions similar to those which have been found to result in the conversion of diiodotyrosine to thyroxine. A new compound, considered to be the lactic acid analogue of thyroxine, was isolated in a nett yield of about 3%.

While one of us (V. M. T.) was abroad, laboratory facilities, enabling the identification of the transformation product of 3:5-diiodo-4-hydroxyphenyllactic acid to be carried out, were kindly provided by the Director, National Institute for Medical Research, London (Dr C. R. Harington, F.R.S.) and Dr W. Engels of the Research Department, Merck and Co., Inc., Rahway, New Jersey. We are further indebted to Mrs R. V. Pitt Rivers of the former institution for the iodine assay and to Dr N. R. Trenner of Merck and Co. for the infra-red examination and report.

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