(28% yield) of IV as yellow colored cubes, m.p. 185–186°, recrystd. from acetone; infrared bands (CCl_4), 1667/92, 1602/12, 1550/24. *Anal.* Calcd. for $C_{25}H_{19}NO$: C, 85.93; H, 5.48; N, 4.01. Found: C, 85.93; H, 5.50; N, 3.89.

1-Phenyl-3-anilino-2-butene-1-one (V).—A sample of this β-anilino-α,β-unsaturated ketone was prepared by the method suggested by Beyer[12] in 43% yield, m.p. 108–110°; λ_{max} (methanol); 244, 353 mμ ($\epsilon \times 10^{-3}$, 13.4, 26.2); λ_{max} (methanol + 0.1 *N* $NaOCH_3$): 244, 353 mμ ($\epsilon \times 10^{-3}$, 13.4, 26.4); λ_{max} (methanol + 0.1 *N* HCl): 244, 307 mμ ($\epsilon \times 10^{-3}$, 7.5, 13.1); infrared bands in $CHCl_3$, 3601/27, 3455/29, 1607/69, 1595/84, 1567/90, 1550/64; in CCl_4, 3060/70, 1609/33, 1596/82, 1570/86, 1559/61; in Nujol, 2930/80, 1617/63, 1589/65, 1549/67, 1522/72.

(12) C. Beyer, *J. prakt. Chem.*, **33**, 393 (1886).

LINCOLN, NEB.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

Model Reactions for the Biosynthesis of Thyroxine. II. The Fate of the Aliphatic Side Chain in the Conversion of 3,5-Diiodophloretic Acid to 3,5,3′,5′-Tetraiodothyropropionic Acid[1]

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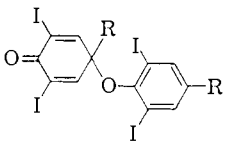
A model reaction for the biosynthesis of thyroxine, the non-enzymic conversion of diiodophloretic acid (diiododesaminotyrosine) to tetraiodothyropropionic acid, has been investigated. The fate of the aliphatic side chain which is eliminated in the course of this reaction has been determined. Hydracrylic acid and dihydracrylic acid are the principal products derived from this side chain. The mechanism of the elimination reaction is discussed.

Introduction

In the biosynthesis of each molecule of thyroxine from two molecules of its precursor 3,5-diiodotyrosine the alanine side chain of one of the two molecules of diiodotyrosine is split off. The fate of this "lost side chain" has been a matter of controversy for many years.

Since the discovery of a simple model reaction for the biosynthesis of thyroxine, the formation of thyroxine in the non-enzymic alkaline incubation of 3,5-diiodotyrosine[3] at 37°, the fate of the alanine side chain in this reaction has been studied by several investigators. Johnson and Tewkesbury[4] postulated that in the conversion of diiodotyrosine to thyroxine a quinol ether intermediate (I) is formed



I, R = $CH_2CH(NH_2)COOH$

which then loses an alanine side chain. They pointed out that this side chain could be lost either as serine or as dehydroalanine (iminopyruvic acid). The latter would hydrolyze to form pyruvic acid and ammonia. According to Harrington[5] serine could be formed not only by the attack of a hydroxyl ion on the quinol ether but also by hydration of the originally formed dehydroalanine.

Johnson and Tewkesbury detected pyruvic acid and ammonia in the incubation mixture but were unable to detect serine. On the other hand, Ohno[6] found serine in the reaction mixture. No experimental data in support of his finding have been reported. Pitt-Rivers[7] who modified von Mutzenbecher's model reaction by substituting N-acetyl-3,5-diiodotyrosine for 3,5-diiodotyrosine and incubating it at *p*H 7.6 found after hydrolysis of the reaction mixture alanine but no serine. Pitt-Rivers pointed out, however, that acetylalanine could have been formed from pyruvic acid and ammonia. In a recent publication Pitt-Rivers and James[8] expressed the opinion that the side chain in this model reaction is split off as hydroxypyruvic acid and acetamide. They were unable to detect hydroxypyruvic acid in the reaction mixture but pointed out that this might be due to the instability of hydroxypyruvic acid.[9] When they incubated a peptide, N-acetyldiiodotyrosyl-ε-N-(α-N-acetyl)-lysine, they could prove the presence of ε-N-hydroxypyruvoyl-α-N-acetyllysine and of acetamide in the reaction mixture. An entirely different model reaction for the biosynthesis of thyroxine was devised by Sela and Sarid.[10] They incubated iodinated polytyrosine and found serine after hydrolysis of the reaction mixture. The incubation was, however, carried out at *p*H 10.2. In this Laboratory an investigation was made with the aim of finding a simpler model reaction for the biosynthesis of thyroxine. Such a reaction was found in the non-enzymic incubation of 3,5-diiodophloretic acid (3,5-diiododesaminotyrosine). This reaction yields 3,5,3′,5′-tetraiodothyropropionic acid (desaminothyroxine) in a yield that is considerably higher than the yield of thyroxine or N-acetylthyroxine obtained in the incubation of diiodotyrosine or N-acetyldiiodotyrosine, respectively.[1] This model reaction was then used to elucidate the fate of the "lost side chain." The reaction mechanism in this case must be assumed to be closely related to the one by which thyroxine is synthesized in von Mutzenbecher's experiment. A knowledge of this

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(2) Visiting Scientist from Osaka City University, Japan.

(3) P. von Mutzenbecher, *Z. physiol. Chem.*, **261**, 253 (1939).

(4) T. B. Johnson and L. B. Tewkesbury, Jr., *Proc. Natl. Acad. Sci., U. S.*, **28**, 73 (1942).

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(7) R. Pitt-Rivers, *Biochem. J.*, **43**, 223 (1948).

(8) R. Pitt-Rivers and A. T. James, *ibid.*, **70**, 173 (1958).

(9) D. B. Sprinson and E. Chargaff, *J. Biol. Chem.*, **164**, 417 (1946).

(10) M. Sela and S. Sarid, *Nature*, **178**, 540 (1956).

mechanism may in turn be of help in the elucidation of the mechanism by which thyroxine is synthesized *in vivo*.

Diiodophloretic acid was incubated essentially as described in an earlier publication.[1] The tetraiodothyropropionic acid formed as well as the bulk of unreacted starting material were removed. The remaining solution was further fractionated by distillation and extraction of the residue with ether. Aliphatic acids deriving from the propionic acid side chain of diiodophloretic acid were found in both the distillate and the ether extract. They were identified by paper chromatographic comparison with authentic samples of these acids.

Methods.—Melting points were taken in capillary tubes and are uncorrected.

Infrared spectra were determined in a Perkin–Elmer recording spectrophotometer, model 21, equipped with sodium chloride optics.

For most paper chromatograms (ascending technique; Whatman paper 3MM) the following solvents[11] were used: (1) ethanol–water–15 *N* ammonia (40:5:1); (2) 1-propanol–water–15 *N* ammonia (40:5:1). The papers were dried at room temperature for at least one hour, then sprayed. An aqueous solution of brom phenol blue containing citric acid[12] (reagent 1) was used for the detection of aliphatic acids; a solution of diazotized N^1,N^1-diethylsulfanilamide[1] (reagent 2) for the detection of phenolic acids. Freshly prepared chromatographic solvents gave almost round spots. Solvents which were several weeks old gave more elongated spots, probably owing to the gradual evaporation of ammonia. Other solvent systems, mentioned in an earlier publication,[1] were used occasionally.

For those reactions in which the *p*H of the reaction mixture had to be kept constant by means of the controlled addition of acid or base, a *p*H-stat (Radiometer, Copenhagen) was used. The acid or base was added below the surface of the stirred reaction mixture through a fine polyethylene tubing. A constant temperature was maintained in these reactions by means of jacketed reaction vessels.

Experimental[13]

3-(*p*-Hydroxyphenyl)-propionic Acid (Phloretic Acid).—A suspension of 53.0 g. (0.32 mole) of *p*-hydroxycinnamic acid (Aldrich Chem. Co.) in 300 ml. of methanol containing 10% water[14] (v./v.) was hydrogenated in the presence of 3 g. of palladium-on-charcoal (10%) at room temperature and slightly above atmospheric pressure. The theoretical amount of hydrogen was taken up in 0.5 hour. The catalyst was removed by filtration and the filtrate evaporated. Recrystallization of the crystalline residue from hot water gave 51.2 g. (95 %) of colorless prisms melting at 128–129°, lit. m.p. 128–129°[1,15] and 129–130°.[16,17]

Incubation of 3-(3,5-Diiodo-4-hydroxyphenyl)-propionic Acid (3,5-Diiodophloretic Acid).—To a suspension of 50.2 g. (0.12 mole) of 3,5-diiodophloretic acid, m.p. 165–167° dec.,[18] in 80 ml. of water enough 1 *N* NaOH (217 ml.) was added to dissolve the acid. The *p*H was adjusted to 7.7 by the addition of 3.5 ml. of 6.4 *N* H_2SO_4, and the resulting opalescent solution was diluted with water to a total volume of 400 ml. This 0.3 *M* solution of 3,5-diiodophloretic acid was incubated in a loosely covered culture bottle at 37° for five days.

The crystalline precipitate of crude sodium 3,5,3′,5′-tetraiodothyropropionate formed (A-1)[19] was removed by centrifugation, then washed on a suction filter with 50 ml. of ice-cold water (4.3 g., 5.5 mmoles). The supernatant and the wash water were combined (A-2) and then acidified with 20 ml. of 10 *N* H_2SO_4. After standing for two days at 4° the precipitate (B-1) consisting essentially of starting material and some 3-iodophloretic acid[1] was filtered and washed with 50 ml. of water (39.8 g.). The combined filtrates (*ca.* 500 ml.) (B-2) were concentrated *in vacuo* to a volume of 80 ml. (C-1).

The aqueous distillate (C-2) was neutralized with 1 *N* NaOH (0.5 mmole), then evaporated *in vacuo* to dryness. A solution of the residue in 3 ml. of water was carefully acidified with 1 *N* HCl, then extracted three times with 10 ml. of ether. The ether extract was dried with sodium sulfate, and the solvent removed *in vacuo*. Chromatography[20] of the residue in solvents 1 and 2 and in 1-butanol–2 *N* ammonia[1] as well as the acetokinase test[21] showed that it was acetic acid.

The concentrate (C-1) was filtered to remove 2.2 g. of solid material (D-1) consisting essentially of starting material and 3-iodophloretic acid. The filtrate (D-2) was extracted with ether in a continuous extractor for 15 hours. The ether extract was dried with sodium sulfate and the solvent evaporated *in vacuo*. A semi-solid residue (E) was obtained (0.30 g.). Chromatography of this residue (solvents 1 and 2) followed by spraying with reagent 1 revealed two intense spots and a weak spot. In a few chromatograms, in which much material was applied, a fourth extremely faint spot could be detected. Comparison with authentic reference substances applied to the same paper and mixed chromatograms showed that the two intense spots were caused by 3-hydroxypropionic acid (hydracrylic acid) and bis-(2-carboxyethyl) ether (dihydracrylic acid).[20] Spraying with reagent 2 revealed only the two weak spots. The one with the lower R_f-value corresponded to 3,5-diiodophloretic acid, the other one to a mixture of 3-iodophloretic acid and phloretic acid.[1]

Methyl 3-Hydroxypropionate (Methyl Hydracrylate) and Bis-(2-carbomethoxyethyl) Ether (Dimethyl Dihydracrylate).—Alkaline hydrolysis of 50 g. (0.7 mole) of 3-hydroxypropionitrile (Eastman Kodak), carried out according to Read[22] except that the multiple extractions with ether were replaced by a continuous extraction for 20 hours, yielded 40 g. of a colorless, viscous product (neut. equiv., calcd. for hydracrylic acid: 90.1; for dihydracrylic acid: 81.1; found: 85.4). Chromatography gave two intense spots (R_f 0.12 and 0.29 in solvent 1; 0.06 and 0.16 in solvent 2) and several faint spots.

A solution of 18 g. of the product in ether was treated with a slight excess of an ethereal solution of diazomethane. The viscous liquid obtained after evaporation of the ether (20.8 g.) was distilled *in vacuo*. Two main fractions (A and B) were obtained. Fraction A (9.1 g.) distilled at 70–82° (11 mm.) ($n^{24}D$ 1.4200–1.4202; fraction B (9.0 g.) at 82–132° (11 mm.) ($n^{24}D$ 1.4296–1.4358). The bulk of fraction A distilled at 73–74° (11 mm.) ($n^{24}D$ 1.4202), the bulk of fraction B at 131–131.5° (11 mm.) ($n^{24}D$ 1.4333). The infrared spectra (smears) of the middle cuts of fraction A (methyl hydracrylate) and of fraction B (methyl dihydracrylate) showed the following characteristic bands: fraction A: 2.92 (hydroxyl), 5.75 (carbonyl of COOR), 9.55 μ (hydroxyl); fraction B: 5.75 (carbonyl of COOR), 8.95 μ

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(12) E. P. Kennedy and H. A. Barker, *Anal. Chem.*, **23**, 1033 (1951).

(13) The microanalyses were made by Dr. W. C. Alford and his associates of the Analytical Service Laboratory of this Institute; the infrared spectra by Mr. H. K. Miller, also of this Institute.

(14) In the presence of absolute methanol, esterification takes place.

(15) C. Stöhr, *Ann.*, **225**, 57 (1884).

(16) J. C. Westfahl and T. L. Gresham, This Journal, **76**, 1076 (1954).

(17) N. Kharasch, S. H. Kalfayan and J. D. Arterberry, *J. Org. Chem.*, **21**, 925 (1956).

(18) J. H. Barnes, E. T. Borrows, J. Elks, B. A. Hems and A. G. Long, *J. Chem. Soc.*, 2824 (1950).

(19) See flow sheet (Fig. 1).

(20) Consult Table I for R_f values.

(21) I. A. Rose, M. Grünberg-Manago, S. R. Korey and S. Ochoa, *J. Biol. Chem.*, **211**, 737 (1954). We thank Dr. E. Stadtman and Dr. T. C. Stadtman, National Heart Institute, Bethesda, Md., for their kind help in carrying out this test.

(22) R. R. Read, "Organic Syntheses," Coll. Vol. I, 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1948, p. 321.

TABLE I

R_f-VALUES

Acid	R_f[a] in solvent 1[b]	R_f[a] in solvent 2[c]
Acetic	0.38	0.22
Propionic	.45	.30
Acrylic	.43	.28
Hydracrylic	.29	.16
Dihydracrylic	.12	.06
Phloretic / 3-Iodophloretic	.42	.31
3,5-Diiodophloretic	.21	.10

[a] Mean values are given in this table. Individual values may differ slightly from these values, depending on the temperature, the age of the solvent, etc. The use of reference substances in each chromatogram is therefore advisable. [b] Ethanol–water–15 *N* ammonia (40:5:1). [c] 1-Propanol–water–15 *N* ammonia (40:5:1)

(aliphatic ether). No ether band was present in the spectrum of fraction A, no hydroxyl band in the spectrum of fraction B; sapon. equivs.: Calcd. for methyl hydracrylate, 104; found for fraction A (middle cut), 107; calcd. for dimethyl dihydracrylate, 95; found for fraction B (middle cut), 95. Fraction B, which was colorless when freshly distilled, turned yellow on long standing and deposited some crystalline material.

A mixture of hydracrylic acid and dihydracrylic acid was also prepared by treating propiolactone with strong alkali. Freshly redistilled propiolactone (6–7 ml., *ca.* 0.1 mole) was added rapidly with stirring to 100 ml. of 5 *N* NaOH (strongly exothermic reaction). The solution was heated overnight (18 hours) to 120° in a loosely covered container, whereby it became more concentrated through the evaporation of some water. Then it was diluted with 100 ml. of water and, after cooling in an ice-bath, acidified with 6 *N* HCl to *p*H 2.5. The solution was evaporated *in vacuo* to dryness (bath temp. <45°). Some Na_2SO_4 was added to the residue which was then extracted three times with anhydrous ether. The combined ether extracts were evaporated *in vacuo*. The colorless, viscous residue consisted of about equal amounts of hydracrylic acid and dihydracrylic acid. The two acids were separated through fractional distillation of their methyl esters as described above.

3-Hydroxypropionic Acid (Hydracrylic Acid). A. By Hydrolysis of Methyl Hydracrylate.—A solution of 0.4 g. (4 mmoles) of methyl hydracrylate (middle cut of fraction A) in 10 ml. of water and 6 ml. of 1 *N* NaOH was permitted to stand overnight at room temperature. Enough 1 *N* HCl was then added with cooling to bring the *p*H to 2.6. The solution was evaporated *in vacuo* (bath temperature <45°). Some Na_2SO_4 was added to the residue which was then extracted three times with anhydrous ether. The combined ether extracts were evaporated *in vacuo*. Hydracrylic acid was obtained as a colorless, somewhat viscous liquid. It was chromatographically pure,[20,23] but elemental analysis showed that it apparently retained traces of water tenaciously (about 2% after drying at room temperature and at a pressure of 0.1 mm. for 5 hours). The infrared spectrum (smear) shows a broad absorption between 2.7 and 4.5 μ caused by the associated hydroxyls of COOH and of CH_2OH, and bands at 5.80 (carbonyl of COOH) and at 9.63 μ (hydroxyl of COOH). There is no ether band in the vicinity of 9 μ. On long standing at room temperature or in the refrigerator, impurities are formed, probably consisting of the ester formed from two molecules of hydracrylic acid[24] and to a lesser extent of the ester formed from three molecules of hydracrylic acid.[25] Elemental analyses of preparations of hydracrylic acid thus contaminated show to high figures for carbon. These preparations can be purified by one or two high vacuum distillations (10^{-4} to 10^{-5} mm., bath temperature 45°).

(23) Hydracrylic acid as well as all other acidic compounds were applied to the paper in the form of their ammonium salts. In the case of hydracrylic acid the addition of ammonia to the free acid must be done carefully with good cooling in order to dissipate the heat of neutralization.

(24) $HO(CH_2CH_2COO)_2H$, R_f 0.37 (solvent 1) and 0.22 (solvent 2).

(25) $HO(CH_2CH_2COO)_3H$, R_f 0.42 (solvent 1) and 0.28 (solvent 2).

B. By Hydrolysis of Propiolactone (Water).—Freshly distilled propiolactone (6–7 ml., *ca.* 0.1 mole) was added slowly (15–20 min.) with stirring to 300 ml. of water. The solution was allowed to stand at room temperature for 16 hours. Evaporation *in vacuo* (bath temperature <45°) yielded chromatographically pure hydracrylic acid.

The same result was obtained when the propiolactone was added to water at 37°.

C. By Hydrolysis of Propiolactone (Alkali).—Freshly distilled propiolactone (6–7 ml., *ca.* 0.1 mole) was added slowly (15–20 min.) with stirring to 300 ml. of 0.5 *N* NaOH. The solution was allowed to stand at room temperature for 3 hours. Then it was brought to *p*H 2.6 by the careful addition with cooling of 5 *N* HCl and evaporated *in vacuo* (bath temperature <45°). Some Na_2SO_4 was added to the residue which was then extracted three times with anhydrous ether. Evaporation of the combined ether extracts *in vacuo* yielded chromatographically pure hydracrylic acid.

The same result was obtained when propiolactone was added to 0.5 *N* NaOH at 37°.

Incubation of 3-Hydroxypropionic Acid (Hydracrylic Acid) at *p*H 7.7–7.8.—A solution of *ca.* 10 mmoles of freshly prepared hydracrylic acid (from 0.7 ml. of propiolactone according to procedure C) in about 4 ml. of water was adjusted to *p*H 7.7 by the addition of 1 *N* NaOH. Enough water was then added to bring the total volume to 15 ml. This solution was incubated at 37° for five days. During this period the *p*H was kept between 7.7 and 7.8 by the slow addition of 1 *N* NaOH (*p*H-stat). The reaction mixture was then cooled, the *p*H brought to 2.6 with 1 *N* HCl and the solution evaporated *in vacuo* (bath temperature <45°). The residue was extracted with ether and the ether extract evaporated in the usual manner. Chromatography of the colorless viscous residue showed that the hydracrylic acid had become contaminated with small amounts of substances tentatively identified as the products formed by esterification of two and of three molecules of hydracrylic acid.[24,25] No dihydracrylic acid was present in the residue.

Incubation of Acrylic Acid at *p*H 7.7–7.8.—Enough 5 *N* NaOH was added slowly and with cooling (ice-bath) to a solution of 3.6 g. (50 mmoles) of freshly redistilled acrylic acid (Monomer–Polymer Labs.) in about 5 ml. of water to bring the *p*H to 7.7. More water was then added to a total volume of 25 ml. This solution was incubated at 37° for five days. During this period the *p*H was kept between 7.7 and 7.8 by the slow addition of 0.1 *N* HCl (*p*H-stat). The *p*H was then brought to 2.6 by the addition of 1 *N* HCl, and the solution evaporated *in vacuo* (bath temperature <45°). Titration of the distillate showed that it contained almost the entire amount of the acrylic acid used in the incubation and that not much polymerization had taken place. Chromatography of the distillate (after neutralization, followed by evaporation, reacidification and repeated extraction with ether in the usual manner) revealed only a single spot of acrylic acid.[20] The residue obtained in the distillation of the acidified incubated solution (see above) was extracted with ether and the ether extract evaporated *in vacuo*. A very small residue was obtained which was dissolved in ethanolic ammonia. The turbid solution (polymers) was filtered and the filtrate concentrated *in vacuo*. Chromatography of the concentrate showed the presence of hydracrylic acid. No dihydracrylic acid was detected.

Incubation of 3-(3,5-Diiodo-4-hydroxyphenyl)-propionic Acid (3,5-Diiodophloretic Acid) in the Presence of Acrylic Acid.—A mixture of 25 g. (60 mmoles) of diiodophloretic acid and 0.22 g. (3 mmoles) of freshly redistilled acrylic acid was incubated and the reaction mixture worked up in the same manner as described above for the incubation of diiodophloretic acid alone. Chromatography of the ether extract of fraction C-2 in solvents 1 and 2 showed two almost equally strong spots having the R_f-values of acetic acid and of acrylic acid.[20] Chromatography of fraction E in the same solvents showed three main spots. The strongest one of these was caused by hydracrylic acid, the other two which were somewhat weaker by dihydracrylic acid and by a mixture of 3-iodophloretic acid and phloretic acid.[20] The latter spot could also be made visible by spraying with reagent 2.

Hydrolysis of Propiolactone at Constant pH.—A series of hydrolyses of propiolactone were carried out at 37° as well as at room temperature, in which the *p*H of the reaction mixture was kept constant at 7.0, 7.7 and 8.0.

The following is a typical example for these reactions. Immediately following the mixing of 0.7 ml. of freshly re-

distilled propiolactone and 15 ml. of water,[26] 5 *N* NaOH was added at such a rate that a constant *p*H was maintained (*p*H-stat). During the addition of the first few drops of alkali the *p*H tended to fluctuate, but soon became stabilized at the desired value. Although the rate of hydrolysis (rate of the addition of alkali) slowed down considerably after 1–2 hours, the reaction was permitted to continue overnight. The solution was then cooled and brought to *p*H 2.6 with 1 *N* HCl. The acidified solution was worked up as described above for the alkaline hydrolysis of propiolactone.

Chromatographic analyses of the reaction products always revealed three spots, no matter whether the hydrolysis was carried out at *p*H 7.0, 7.7 or 8.0, at room temperature or at 37°. The most intense one of these spots was caused by hydracrylic acid[20]; a somewhat weaker spot and a still weaker one were caused by two unidentified substances believed to be the products formed by esterification of two and of three molecules of hydracrylic acid.[24,25]

Bis-(2-carboxyethyl) Ether (Dihydracrylic Acid).—A suspension of 1.3 g. (6.8 mmoles) of dimethyl dihydracrylate (middle cut of fraction B) in 20 ml. of water and 15 ml. of 1 *N* NaOH was refluxed for 1 hour. After cooling to room temperature the solution was acidified with 1 *N* HCl and evaporated *in vacuo* to dryness. The residue was extracted with ether and the ether extract dried with sodium sulfate and evaporated. The yellow, crystalline residue (1.1 g.) was taken up in a small amount of ether. Some insoluble, yellow material was removed by filtration. After evaporation of the filtrate and crystallization of the residue from chloroform–benzene, colorless plates, m.p. 62–63.5°, were obtained which were chromatographically pure.[20] The infrared spectrum (KBr) shows a broad band between 2.7 and 4.5 μ (associated COOH) and bands at 5.85 (carbonyl of COOH), 8.99 (aliphatic ether) and 10.57 μ (hydroxyl of COOH). The crystals tend to soften on standing at room temperature for long periods of time.

Anal. Calcd. for $C_6H_{10}O_5$: C, 44.44; H, 6.22. Found: C, 44.62; H, 6.11.

Acid Treatment of 3-Hydroxypropionic Acid (Hydracrylic Acid).—3-Hydroxypropionic acid (0.15 g.) freshly prepared from its methyl ester was dissolved in 5 ml. of ether saturated with 2 *N* H_2SO_4. The solution was refluxed for 1 hour, then evaporated. Paper chromatography of the residue (solvents 1 and 2) showed that a trace of acrylic acid had been formed but no dihydracrylic acid.[20]

Alkali Treatment of 3-Hydroxypropionic Acid (Hydracrylic Acid).—A solution of sodium 3-hydroxypropionate was prepared by mixing 3.1 g. (30 mmoles) of methyl 3-hydroxypropionate (middle cut of fraction A) with a solution of 1.4 g. (35 mmoles) of sodium hydroxide in 4.5 ml. of water and letting this mixture stand overnight at room temperature. This solution was then treated with alkali in exactly the same manner as 3-hydroxypropionitrile.[27] Chromatography of the colorless, viscous product obtained after evaporation of the ether extract revealed two unidentified faint spots and two strong spots caused by hydracrylic acid and dihydracrylic acid.[20]

Results and Discussion

Aliphatic acids formed in the course of the incubation of diiodophloretic acid were found in fractions C-2 and E (Fig. 1). Fraction C-2 contained a small amount of acetic acid (9% of the theory, based on the amount of sodium tetraiodothyropropionate formed). The bulk of the aliphatic acids deriving from the propionic acid side chain of diiodophloretic acid were non-volatile and therefore found in fraction E (roughly two-thirds of the theory,[28] based on the amount of sodium tetraiodothyropropionate formed).

On the basis of the findings of previous investigators it was expected that either acrylic acid or hydracrylic acid, the desamino analogs of dehydroalanine and of serine, respectively, would be present in the reaction mixture from the incubation of diiodophloretic acid. Propionic acid (desaminoalanine) was also considered a possibility since the enzymic formation of alanine from diiodotyrosine had been reported in the literature.[29] Chromatography and mixed chromatography established without doubt that neither acrylic acid nor propionic acid were present in fractions C-2 and E. When a small amount of acrylic acid was incubated together with a large amount of diiodophloretic acid, the former was easily detected in fraction C-2 together with acetic acid. This experiment and other experiments in which acrylic acid was incubated alone, both aerobically and anaerobically, clearly established that the amount of polymerization taking place during the incubation was insignificant and did not prevent the detection of even a very small amount of acrylic acid.

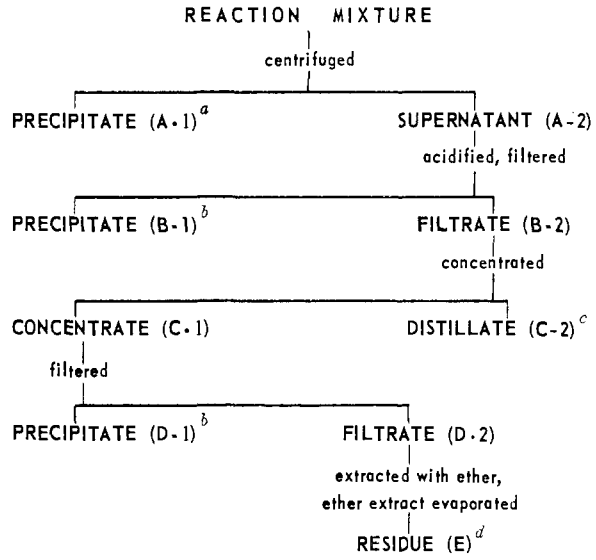


Fig. 1.—Flow sheet for the fractionation of the reaction mixture resulting from the incubation of 3,5-diiodophloretic acid: [a] sodium 3,5,3′,5′-tetraiodothyropropionate; [b] 3,5-diiodophloretic acid and 3-iodophloretic acid; [c] aqueous solution of acetic acid; [d] mixture of hydracrylic acid and dihydracrylic acid, contaminated with phloretic acid and iodinated phloretic acids.

In order to determine whether one of the two aliphatic acids found in fraction E was hydracrylic acid an authentic sample of this acid was required. The preparation of a mixture of essentially pure hydracrylic acid and water by alkaline hydrolysis of 3-hydroxypropionitrile is described in reference 22. In our hands this procedure yielded a mixture of about equal parts of hydracrylic acid and of an unknown acid. The two acids proved to be chromatographically identical with the two aliphatic acids in fraction E. The mixture of the two acids was treated with diazomethane and the two methyl esters thus formed separated by fractional distillation, then individually hydrolyzed. Infrared and elemental analyses of the acids obtained as well as infrared analyses and saponification equivalents of the corresponding methyl esters showed that the unknown acid was bis-(2-carboxyethyl) ether (di-

(26) In some experiments, 30 ml. of water was used.

(27) See under preparation of methyl 3-hydroxypropionate and bis-(2-carbomethoxyethyl) ether.

(28) A more accurate estimation of the yield is not possible since the side chain acids in fraction E were contaminated with phloretic acid, mono- and diiodophloretic acid and traces of other impurities.

(29) J.-G. Ljunggren, *Acta Chem. Scand.*, **11**, 1072 (1957).

hydracrylic acid), an acid that has not been described in the literature.[30]

In order to determine whether it had been formed during the alkaline hydrolysis of 3-hydroxypropionitrile or rather during the subsequent continuous ether extraction of the reaction product in the presence of acid, hydracrylic acid was treated with acid as well as with alkali under the conditions prevailing during the treatment of 3-hydroxypropionitrile.[27] These experiments showed that the dihydracrylic acid had been formed under the influence of alkali, not of acid. In another experiment propiolactone was added to strong alkali (5 *N* NaOH) and the reaction mixture heated to 120°. Here, too, a mixture of hydracrylic and dihydracrylic was formed.[31] The simplest way to prepare pure hydracrylic acid, free from dihydracrylic acid and from other contaminants,[24,25] is to hydrolyze propiolactone either with dilute alkali or with water under the mild conditions described in the Experimental part. Slightly contaminated hydracrylic acid can be purified by high vacuum distillation without dehydration (formation of acrylic acid, of dihydracrylic acid or of esters[24,25]) taking place.

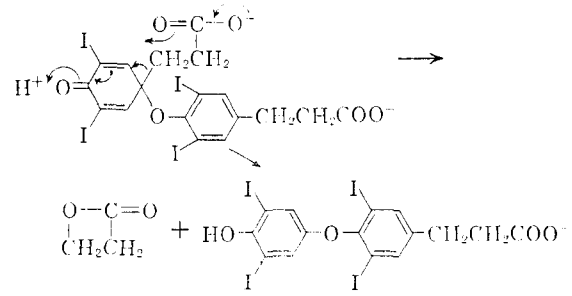
Since dihydracrylic acid is formed from hydracrylic acid under the influence of alkali, it was suspected that the dihydracrylic acid found in the reaction mixture from the incubation of diiodophloretic acid had also be enformed from hydracrylic acid during the incubation.[32] However, when hydracrylic acid was incubated under the same conditions (*p*H 7.7–7.8, 37°, 5 days), no dihydracrylic acid was formed. It is therefore unlikely that hydracrylic acid is the precursor of the dihydracrylic acid.

Another possibility was then considered, *viz.*, that in the incubation experiment the propionic acid side chain is eliminated in the form of a common precursor of hydracrylic acid and of dihydracrylic acid. If acrylic acid were the common precursor, one would have to assume that

$$H_2C{=}CHCOOH \xrightarrow{HOH} HOCH_2CH_2COOH$$
$$H_2C{=}CHCOOH \xrightarrow{ROH} ROCH_2CH_2COOH$$
$$R = CH_2CH_2COOH$$

it is completely converted to hydracrylic acid and dihydracrylic acid since it was not present in the reaction mixture. When acrylic acid was incubated under the same conditions (*p*H 7.7–7.8, 37°, 5 days) only a trace of hydracrylic acid was formed but no dihydracrylic acid. Practically all the starting material was recovered unchanged.

The possibility that propiolactone is the common precursor was also considered. Johnson and Tewkesbury's mechanism for the synthesis of thyroxine[4,5,33] can be modified so that the aliphatic side chain is split off neither as an unsaturated compound (acrylic acid) nor as a hydroxylated compound (hydracrylic acid) but as a cyclic compound (propiolactone). The chemical literature abounds



in examples of similar cyclic mechanisms.[34] The close proximity of the oxygens of the side chain to the potential carbonium ion next to the quinol ring makes such a mechanism attractive. In the aqueous medium of the incubation mixture, the propiolactone formed would hydrolyze to form hydracrylic acid. The formation of dihydracrylic acid could then be explained by an attack of the hydracrylic acid formed by this hydrolysis on a second molecule of propiolactone. It has been shown that the propiolactone ring can be opened in two different ways depending on the *p*H of the medium, *viz.*, by acyl–oxygen fission or by alkyl–oxygen fission.[35,36] Only the latter type of fission could lead to dihydracrylic acid, while under the conditions of our incubation experiment the former appears more likely. In order to determine whether or not under these conditions dihydracrylic acid is formed from propiolactone the latter was hydrolyzed at *p*H 7.7–7.8. Hydracrylic acid and esters formed from two and from three molecules of hydracrylic acid[24,25] were the only reaction products; no dihydracrylic acid was formed.

It appears then that under the conditions of the incubation experiment hydracrylic acid does not react with itself, with acrylic acid nor with propiolactone to form dihydracrylic acid. There remains, however, another possible reaction mechanism for the formation of dihydracrylic acid from propiolactone. In the course of the hydrolysis of the latter in the presence of the quinol ether intermediate (I), a concerted push-pull mechanism would result in the formation of dihydracrylic acid. Although this mechanism lacks experimental proof it should be considered since various other possible reaction mechanisms have been excluded.

Pitt-Rivers and James[8] suggested that the first step in the conversion of diiodotyrosine to thyroxine may be an oxidation of the methylene group

(30) A sodium salt of the composition $C_6H_8O_5$ was prepared by J. Wislicenus, *Ann.*, **166**, 3 (1873); *cf.* O. Meister, *Ber.*, **3**, 808 (1870). It can be concluded from the method of its preparation as well as from its reactions that it is the disodium salt of bis-(2-carboxyethyl) ether. Wislicenus named the parent acid dihydracrylic acid.

(31) W. A. Drushel and W. H. T. Holden, *Am. J. Sci.*, **40**, 511 (1915), found that "condensation products" are formed when alkaline solutions of hydracrylic acid are evaporated. These condensation products were not further investigated. They must have consisted essentially if not entirely of dihydracrylic acid.

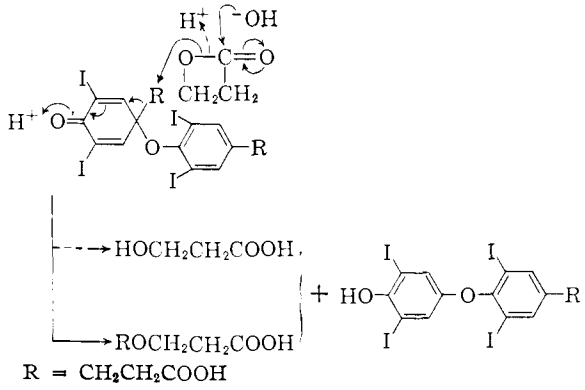
(32) The starting *p*H in that incubation was 7.7, the final *p*H 7.8.

(33) T. Matsuura and H. J. Cahnmann, Abstracts 134th Meeting of the American Chemical Society, September 1958, Chicago, Ill., p. 11-O.

(34) In the synthesis of thyroxine instead of tetraiodothyropropionic acid various cyclic intermediates with either an oxygen or a nitrogen in the heterocycle can be conceived. This does, however, not necessarily mean that such an intermediate would open in the same manner as propiolactone (see below) to form a hydroxylated compound and an ether.

(35) T. L. Gresham, J. E. Jansen, F. W. Shaver, J. T. Gregory and W. L. Beears, THIS JOURNAL, **70**, 1004 (1948).

(36) F. A. Long and M. Purchase, *ibid.*, **72**, 3267 (1950).



adjacent to the aromatic ring to –CH(OH)–. They based this suggestion on a model reaction in which tetraiodothyroacetic acid is formed when 3,5-diiodo-4-hydroxyphenylacetic acid is incubated. The reaction mixture contained glyoxylic acid, the formation of which is easily explained by an oxidation of the starting material to 3,5-diiodo-4-hydroxyphenylglycolic acid, followed by a hydroxylation of the aliphatic side chain according to Johnson and Tewkesbury's mechanism. However, the choice of the acetic acid analog of diiodotyrosine as a model for the study of the fate of the "lost side chain" in the synthesis of thyroxine is not a fortunate one. In contrast to diiodotyrosine and to its propionic acid analog which we chose as a model, the acetic acid analog contains an active methylene group that is very susceptible to oxidation. While 3-(3,5-diiodo-4-hydroxyphenyl)-hydracrylic acid could not be detected with certainty in the reaction mixture from the incubation of diiodophloretic acid,[37] the homologous 3,5-diiodo-4-hydroxyphenylglycolic acid is present in fairly large amounts in the reaction mixture from the incubation of 3,5-diiodo-4-hydroxyphenylacetic acid.[1] Furthermore, in order to explain the formation of hydracrylic acid from 3-(3,5-diiodo-4-hydroxyphenyl)-hydracrylic acid one would have to assume that the aliphatic side chain is eliminated as a carbanion $^{-}CH(OH)CH_2COOH$, a reaction mechanism that is difficult to conceive. The much more plausible mechanism of Johnson and Tewkesbury would lead not to hydracrylic acid but to the semi-aldehyde of malonic acid. Decarboxylation of this unstable compound followed by oxidation would yield acetic acid.[38] The small amount of acetic acid found in fraction C-2 may have been formed in this manner since hydracrylic acid does not give rise to even traces of acetic acid under the conditions of the incubation experiment. Only a small fraction, if any, of the incubated diiodophloretic acid follows this pathway. Reduction of an aliquot of fraction C-2 by diphosphopyridine nucleotide in the presence of alcohol dehydrogenase from horse liver showed that only a minute amount (about 0.01 mmole in the total fraction) of a carbonyl compound reducible by this system, presumably acetaldehyde, was present.

It can be concluded from the present model experiment that in the synthesis of thyroxine from diiodotyrosine the alanine side chain which is eliminated is probably converted to a hydroxylated compound (either serine or hydroxypyruvic acid), not to alanine or dehydroalanine (pyruvic acid and ammonia). It is not likely that the elimination is preceeded by a hydroxylation of the side chain.

(37) A small amount of an unknown substance was found which may be this acid. It had R_f 0.05 in 1-butanol–2 N ammonia and 0.11 in 1-butanol–dioxane–ammonia.[1] It does not seem to be 3,5-diiodo-4-hydroxybenzoic acid since it has a somewhat different R_f-value in 1-butanol–pyridine–water.[1] It gives a brownish-yellow spot on paper chromatograms when sprayed with reagent 2, which indicates that an oxygen function is probably present on the carbon atom adjacent to the aromatic ring.[1]

(38) *Cf.* H. D. Dakin, *J. Biol. Chem.*, **5**, 409 (1909).

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[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

Model Reactions for the Biosynthesis of Thyroxine. III. The Synthesis of Hindered Quinol Ethers and their Conversion to Hindered Analogs of Thyroxine[1,2]

BY TERUO MATSUURA[3] AND H. J. CAHNMANN

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Model reactions are presented for the biosynthetic scheme (equations 1-3) proposed by Johnson and Tewkesbury for the formation of thyroxine from diiodotyrosine. Sterically hindered analogs of the quinol ether III have been synthesized in a sequence of free radical reactions and then converted to the corresponding analogs of thyroxine. Some of the properties of the quinol ethers and of the analogs of thyroxine are described.

The mechanism of the reaction in which thyroxine is formed from diiodotyrosine *in vivo* or *in vitro*[4] is still obscure. Various hypotheses concerning this mechanism have been proposed.[5] The present report deals with the mechanism that was first suggested by Johnson and Tewkesbury[6] and elaborated by Harington.[7] This mechanism is based on the extensive studies of Pummerer and his co-workers[8a–k] who have found that many oxidations of

(1) Paper II, H. J. Cahnmann and T. Matsuura, THIS JOURNAL, **82**, 2050 (1960).

(2) A preliminary report of this work has been presented at the 134th Meeting of the American Chemical Society, September, 1958, Chicago, Ill.

(3) Visiting Scientist from Osaka City University, Japan.

(4) P. von Mutzenbecher, *Z. physiol. Chem.*, **261**, 253 (1939).

(5) For references *cf.* paper I of this series.[1a]

(6) T. B. Johnson and L. B. Tewkesbury, Jr., *Proc. Natl. Acad. Sci. U. S.*, **28**, 73 (1942).

(7) C. K. Harington, *J. Chem. Soc.*, 193 (1944).

(8) (a) R. Pummerer, H. Puttfarcken and P. Schopflocher, *Ber.*, **58**, 1808 (1925); see also (b) R. Pummerer and F. Frankfurter, *ibid.*, **47**, 1472 (1914); (c) **52**, 1416 (1919); (d) R. Pummerer and E. Cherbuliez, *ibid.*, **47**, 2957 (1914); (e) **52**, 1392 (1919); (f) R. Pummerer, *ibid.*, **52**, 1403 (1919); (g) R. Pummerer, D. Melamid and H. Puttfarcken, *ibid.*, **55**, 3116 (1922); (h) R. Pummerer and A. Rieche, *ibid.*, **59**, 2161 (1926); (i) R. Pummerer and F. Luther, *ibid.*, **61**, 1102 (1928); (j) R. Pummerer, G. Schmidutz and H. Seifert, *Chem. Ber.*, **85**, 535 (1952); (k) R. Pummerer and I. Veit, *ibid.*, **86**, 412 (1953).