[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TOLEDO]

3,5-Diiodo-4-aminophenylalanine: A Nitrogen Analog of Diiodotyrosine

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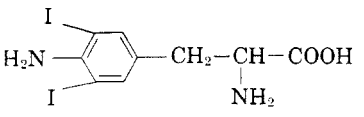
p-Aminophenylalanine and its α-acetylated derivative have been iodinated and the positions which the iodine atoms assumed on the ring have been proved by oxidative degradation.

For its possible biological interest, a nitrogen analog of diiodotyrosine, 3,5-diiodo-4-aminophenylalanine, has been synthesized.



It was readily established that, unlike the case of tyrosine, iodination of *p*-aminophenylalanine in cold alkaline solution was not fruitful, nor was iodination in hot acid solution.[1] Therefore, *p*-toluidine was used in preliminary experiments with the hope, which later proved well founded, that the similarity of structure would enable the conditions for iodination of the one compound to be applied to the other. *p*-Toluidine was first iodinated by Michael and Norton using iodine monochloride,[2] who reported a black precipitate at the beginning of the reaction that could be removed by repeated crystallization and which was minimized by use of a large excess of the toluidine. Later, Wheeler[3] reported a similar experience using iodine and calcium carbonate and only a small excess of toluidine. It has now been found that by avoiding an excess of iodine monochloride in the reaction mixture smooth iodination takes place without any appearance of dark impurities. The rate of addition was critical. It had to be slow enough to prevent the presence in the reaction of excess iodine monochloride. Temperature was not critical. There appeared to be no advantage in using an ice-bath. The results with *p*-aminophenylalanine were entirely analogous. However in this case the product consisted of the hydrochloride of the amino acid and not the free diiodinated amine as in the case of toluidine.

The hydrochloride of diiodo-*p*-aminophenylalanine was stable at room temperature, losing no hydrogen chloride after long standing in a desiccator over soda lime. It came down as a heavy yellow granular precipitate, consisting of spherules, from the iodinating solution. On recrystallization from dilute hydrochloric acid, a cottony precipitate of short needles was formed. It was a very light cream color. Seeding of the iodinating solution with the needleform crystals sometimes resulted in their appearance in the first instance instead of the heavier, sandy precipitate.

The free amino acid was readily prepared from the hydrochloride.

The diiodo amino acid gave a positive ninhydrin test, best in a buffer at *p*H 4; it also gave the *beta*-naphthol test when dissolved in concentrated sulfuric acid, and the solution subsequently was diluted and diazotized. The amino acid is only very slightly soluble in water; the hydrochloride somewhat more soluble; the sodium salt sufficiently insoluble as to be readily crystallized from concentrated aqueous solution.

Although the presumption that the iodine atoms have entered the ring *ortho* to the amino group is overwhelming, the point was settled positively by oxidative degradation. For this purpose the iodinated amino acid was exhaustively acetylated, followed by oxidation with buffered permanganate. Wheeler,[3] who oxidized 3,5-diiodo-4-acetaminotoluene in like manner, obtained only the diiodoaminobenzoic acid, although in a similar oxidation of 3-iodo-4-acetaminotoluene hydrolysis of the acetyl group had not taken place. In the present instance, only the monoacetylated 3,5-diiodo-4-aminobenzoic acid was isolated from the oxidation reaction. However, this compound decomposed without melting, while the unacetylated compound melted with decomposition at a very high temperature that varied considerably with the rate of heating. Fortunately a diacetyl derivative could readily be prepared for comparison with an authentic sample for positive identification.

To obtain the required amounts of *p*-aminophenylalanine, several syntheses were investigated. The compound was prepared originally by Erlenmeyer[4] by nitrating phenylalanine and reducing the nitro compound. Although high yields were reported and in fact verified, the products contained impurities, such as un-nitrated phenylalanine and possibly isomeric nitro derivatives that could be entirely removed only with considerable crystallization losses.

Another approach started with the reaction of *p*-nitrobenzaldehyde and aceturic acid to prepare *alpha*-acetamino-*p*-nitrocinnamic acid by the

(1) Block and Powell, *J. Am. Chem. Soc.*, **65**, 1430 (1943).
(2) Michael and Norton, *Am. Chem. J.*, **1**, 263 (1880).
(3) Wheeler and Liddle, *Am. Chem. J.*, **42**, 449 (1909).
(4) Erlenmeyer and Lipp, *Ann.*, **219**, 213 (1883).

method of Dakin.[5] A similar synthesis using glycine anhydride has been reported,[6] as has also the use of *p*-nitrobenzaldehyde with hippuric acid.[7]

Although the desired amino acid resulted readily by the reduction of the cinnamic acid derivative with hydriodic acid, the use of catalytic reduction under conditions which readily yield phenylalanine[8] gave as the principal product a compound with reduced nitro group only: *alpha*-acetamino-4-aminocinnamic acid.

A more practical approach to the synthesis appeared in the alkylation of acetamidomalonic ester as first reported by Burckhalter,[9] and precise directions for synthesizing *p*-aminophenylalanine on a 1 mole scale has been worked out, using this intermediate. In the earlier work the nitro group was reduced prior to hydrolysis of the malonic ester; here this order has been reversed to take advantage of the greater ease of isolating the more soluble amino acid from a nonionic medium.

EXPERIMENTAL[10]

Ethyl 2-acetamido-2-carbethoxy-3-p-nitrophenylpropionate. A solution of 225 g. of ethyl acetamidomalonate and 23 g. of sodium in 2700 ml. of dry ethanol was heated to boiling in a 5-l. flask equipped with a reflux condenser and an efficient stirrer. The source of heat was removed and *p*-nitrobenzyl bromide (216 g.) in 350 ml. of dry dioxane, heated sufficiently to effect complete solution, was poured into the ethanolic solution all at once while stirring strongly. Separation of a bulky, gelatinous mass began immediately. The reaction mixture was maintained at the reflux temperature for 1.2 hours, cooled, and refrigerated overnight. The filtered precipitate was suspended in 1.5 l. of water to dissolve sodium bromide, filtered, and dried. The product weighed 295 g. (84%) and melted at 195–196°. It was used without further purification. Recrystallization from glacial acetic acid raised the melting point to 196–197°.

p-Nitrophenylalanine. The above ester (105.5 g., 0.3 mole) was dissolved in 250 ml. of acetic acid by heating to the reflux temperature and 175 ml. of 48% hydrobromic acid was added. The solution was boiled under reflux for 5.5 hours, cooled, and refrigerated. Yield, 67 g. (77%) of the hydrobromide of *p*-nitrophenylalanine, dried in a porcelain dish heated on a steam-cone. Re-use of the filtrate for the hydrolysis of a like amount of the ester, adding 50 ml. of hydrobromic acid after the ester had dissolved, resulted in yields as high as 95%, m.p. (dec.) 243°.

Anal. Calc'd for $C_9H_{11}BrN_2O_4$: Br, 27.47. Found: Br, 27.76.

The *hydrobromide* (70 g.) was dissolved in 225 ml. of water, treated with decolorizing carbon, filtered hot, and neutralized with ammonium hydroxide. Yield, 45 g. (90%); m.p. (dec.) 245°. A further amount could be obtained by evaporation of the filtrate.

p-Aminophenylalanine. *p*-Nitrophenylalanine (35 g.) was dissolved in 140 ml. of water at 60° by the addition of the minimum amount of concentrated potassium hydroxide solution. A 5% palladium catalyst[11] (6 g.) was added and the solution was hydrogenated under an initial pressure of 45 lbs. The catalyst was removed by filtration and washed with two 10-ml. portions of hot water. The filtrate, to which the washings had been added, was heated to boiling and acidified with acetic acid. After refrigeration overnight, the yield of separated product was 28 g. (85%) m.p. (dec.) 264°, heated rapidly from 150°.

4-Amino-3,5-diiodophenylalanine hydrochloride. A solution of 5 g. of *p*-aminophenylalanine in 75 ml. of water and 2.5 ml. of hydrochloric acid was stirred at room temperature. To this was added dropwise, during 30–45-min., 39 ml. of a solution made by diluting with water 40 g. of iodine monochloride and 30 ml. of hydrochloric acid to 180 ml. During the addition a sandy yellow precipitate appeared, but to complete the reaction the mixture was refrigerated for at least 6 hours after the completion of the addition. The crude material weighed 10.75 g. and melted with decomposition at 205°. It was recrystallized from dilute hydrochloric acid (8 ml. diluted to 100 ml. with water, using 15 ml. for each gram), decolorizing with the minimum amount of sodium bisulfite, preheating the solution to boiling, and cooling rapidly to avoid subjecting the iodine compound to prolonged heat. The amino acid hydrochloride crystallized rapidly as a cream-colored, cottony precipitate. Recovery: 95%.

Anal. Calc'd for $C_9H_{11}ClI_2N_2O_2$: Cl, 7.58. Found: Cl, 7.75.

The free amino acid was prepared by dissolving the hydrochloride (1 g.) in 150 ml. of 80% ethanol by means of a few ml. of a concentrated aqueous potassium hydroxide solution, and acidifying at the boiling point with acetic acid; m.p. (dec.) 260–262°. Unlike the hydrochloride, liquefaction was not preceded by the appearance of violet fumes of iodine.

Anal. Calc'd for $C_9H_{10}I_2N_2O_2$: C, 25.00; H, 2.32; I, 58.80. Found: C, 24.91; H, 2.52; I, 58.64.

Acetyl-p-nitrophenylalanine. *p*-Nitrophenylalanine (11.5 g.) was dissolved in 50 ml. of 2 *N* sodium hydroxide. While stirring the solution in an ice-bath, 6.3 ml. of acetic anhydride was slowly added. After standing several hours the acetyl derivative was precipitated by the addition of hydrochloric acid. The crude material was crystallized from 100 ml. of 50% acetic acid. The yield was 12.2 g. (89%), m.p. 203.5–204°.

Anal. Calc'd for $C_{11}H_{12}N_2O_5$: C, 52.38; H, 4.79; N, 11.11. Found: C, 52.62; H, 4.89; N, 11.41.

Acetyl-p-aminophenylalanine. The above nitro compound (10 g.) was dissolved in 75 ml. of hot ethanol, 3 g. of a 5% palladium catalyst was added, and hydrogen was admitted at an initial pressure of 45 lbs. After removing the catalyst, and washing it with hot water, the ethanol and washings were distilled off under a vacuum, and the syrupy residue was kept in the refrigerator for several days, after which it was triturated with a little ethanol, filtered and dried. Yield, 7.7 g. (87%) of a cream-colored solid, m.p. 173–175°.

Anal. Calc'd for $C_{11}H_{14}N_2O_3$: C, 59.45; H, 6.34; N, 12.61. Found: C, 59.55; H, 6.37, N, 12.63.

An *acetyl* derivative was prepared from acetic anhydride in aqueous solution; m.p. 210.5–211°.

Anal. Calc'd for $C_{13}H_{16}N_2O_4$: C, 59.08; H, 6.10; N, 10.61. Found: C, 58.88; H, 6.05; N, 10.67.

Acetyl-4-amino-3,5-diiodophenylalanine. Acetyl-*p*-aminophenylalanine (11 g.) was iodinated in the same manner as the unacetylated compound. After 24 hours in the refrigerator, 22 g. (93%) of a product melting at 196.5–198.5° was obtained. For purification it was suspended in cold water (20 ml. per gram), dissolved with the minimum amount of ammonium hydroxide, treated with decolorizing carbon, reprecipitated with acetic acid, and finally crystallized from 50% acetic acid (16.5 ml. per gram). Over-all recovery: 78%. M.p., 201–202°.

Anal. Calc'd for $C_{11}H_{12}I_2N_2O_3$: C, 27.87; H, 2.55; N, 5.91; I, 53.55. Found: C, 27.88; H, 2.61; N, 5.93; I, 53.34.

Diiodo-p-toluidine. *p*-Toluidine (5.35 g.) dissolved in 70 ml. of water and 7 ml. of hydrochloric acid was stirred at room temperature while 17 g. of iodine monochloride in

(5) Dakin, *J. Biol. Chem.*, **82**, 439 (1929).
(6) Ueda, *Ber.*, **61**, 146 (1928).
(7) Bergmann, *J. Am. Chem. Soc.*, **74**, 4947 (1952).
(8) Blatt, *Org. Syntheses*, Coll. Vol. **2**, 491 (1943).
(9) Burckhalter and Stephens, *J. Am. Chem. Soc.*, **73**, 56 (1951).
(10) Melting points are corrected.
(11) Horning, *Org. Syntheses*, Coll. Vol. **3**, 685 (1955).

72 ml. of water and 18 ml. of hydrochloric acid was added very slowly (1 drop in 2 secs.). The reaction mixture was stirred for 15 mins. after the completion of the addition, then warmed to 60°, at which temperature the yellowish color disappeared, leaving a precipitate that was white with only a slight pinkish cast. After standing overnight the product was collected by filtration. Yield: 15 g. (85%), m.p. 126°.

4-Diacetylamino-3,5-diiodobenzoic acid. *p*-Aminobenzoic acid was iodinated under the same conditions as *p*-toluidine, except that the iodine monochloride was added all at once, and the reaction was maintained at 75° for 2 hours. The product was leached twice with generous amounts of boiling ethanol. Yield: 85%. Further purification was effected by recrystallizing the sodium salt. M.p. (dec.) 361°, heated at the rate of 10° per minute from 335°. According to the literature no melting takes place by 350°.[3] One g. was treated with 5 ml. of acetic anhydride and two drops of sulfuric acid and maintained at 80° for 30 mins. The solution was added to boiling water and, when the odor of acetic anhydride had disappeared, the precipitate was collected and crystallized from ethanol, m.p. 200–201°.

Anal. Calc'd for $C_{11}H_9I_2NO_4$: C, 27.91; H, 1.90; I, 53.66. Found: C, 28.09; H, 2.08; I, 53.47.

Monoacetylamino-3,5-diiodobenzoic acid. (*A*). *From 4-diacetylamino-3,5-diiodobenzoic acid.* The diacetyl compound (4 g.) was dissolved in 25 ml. of 10 percent potassium hydroxide, heated to boiling for 15 min., cooled, and acidified with hydrochloric acid. The filtered precipitate was recrystallized several times from dilute ethanol. Heated at 10° per minute from 245°, iodine fumes were noticeable at 280°. As heating was continued, tar condensed in the upper part of the capillary, but the residue did not melt.

Anal. Calc'd for $C_9H_7I_2NO_3$: I, 58.89. Found: I, 58.69.

(*B*). *From iodinated p-aminophenylalanine.* Diiodo-*p*-aminophenylalanine or its *alpha*-acetylated derivative (10 g.) was dissolved in 50 ml. of acetic anhydride and 3 drops of sulfuric acid were added. After 30 mins. on the steam-bath, excess acetic anhydride was destroyed with boiling water, and the gummy product was cooled, separated, and allowed to stand until hard. The crude material (9 g.) was pulverized and added to 24 g. of magnesium sulfate and 21 g. of potassium permanganate in 500 ml. of water. The reaction mixture was stirred on the steam-bath until colorless, treated with sulfur dioxide to the disappearance of manganese dioxide, and the precipitate was separated. This was dissolved in sodium bicarbonate solution to remove darker impurities, precipitated with acid, and crystallized repeatedly from dilute ethanol, using decolorizing charcoal. There was obtained 1 g. of a product that resembled the monoacetyl compound above, although in a capillary, iodine fumes were given off beginning at 260°, indicating that the compound had not yet been completely purified. However acetylation under the conditions used for preparing the diacetyl acid from the unacetylated compound gave a product that melted at 191°. Mixed with the authentic 4-diacetylamino-3,5-diiodobenzoic acid, melting took place at 194°.

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