[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGY AND VITAL ECONOMICS, UNIVERSITY OF ROCHESTER SCHOOL OF MEDICINE AND DENTISTRY]

Synthesis of 2,5-Dihydroxyphenylalanine

BY JOHN P. LAMBOOY

Theoretical consideration of the pathway of phenylalanine and tyrosine metabolism in inborn or ascorbic acid deficiency induced alcaptonuria has frequently included 2,5-dihydroxyphenylalanine as an intermediate. Hirai[1] reported its synthesis in 1927 but the yield which he obtained was poor and it now appears that the identity of his product may be subject to question. Recently Neuberger[2] reported the synthesis of this amino acid by Hirai's procedure and by a procedure similar to one of those reported here. Our two products are clearly identical and very unlike that obtained by Hirai. While this article must, in view of Neuberger's recent article, be considered primarily as confirmatory in nature, it is felt that our superior yields and considerably more convenient procedure will be of interest to investigators in the field of metabolism.

Essentially, the synthetic procedures studied were the condensations of 2,5-dihydroxybenzaldehyde or 2,5-diacetoxybenzaldehyde with either acetylglycine or benzoylglycine in the presence of acetic anhydride and sodium acetate. The products thus formed were converted directly to the amino acid by one-step reduction and hydrolysis with hydriodic acid and red phosphorus.

In the condensation between 2,5-dihydroxybenzaldehyde or 2,5-diacetoxybenzaldehyde and acetylglycine Neuberger's results differ somewhat from ours. We both obtain two compounds, a high melting, and colorless compound shown by Neuberger to be 2-keto-3-acetamino-6-acetoxycoumarin and a lower melting compound which was the true azlactone. Neuberger's optimum reaction conditions for the formation of almost exclusively the azlactone differ very slightly from ours which led to the almost exclusive formation of the acetoxycoumarin. Both compounds are converted to the desired amino acid by reduction and hydrolysis.

Our primary concern was to outline a procedure by which relatively good yields of the amino acid could be obtained with a minimum of effort. Since purification procedures led to relatively large losses of material the use of crude intermediates was investigated. This led to a considerable increase in the yield of amino acid based on starting material 2,5-dihydroxybenzaldehyde.

The best procedure was found to be the conversion of 2,5-dihydroxybenzaldehyde to 2,5-diacetoxybenzaldehyde. This material in the crude form was converted to 2-phenyl-4-(2,5-diacetoxybenzal)-5-oxazolone which in turn, in its crude form was converted to 2,5-dihydroxyphenylalanine of a high degree of purity with an over-all yield of 26% based on starting material 2,5-dihydroxybenzaldehyde. The triacetyl derivative was prepared for purposes of identification through its sharp melting point.

(1) Hirai, *Biochem. Z.*, **189**, 88 (1927).

(2) Neuberger, *Biochem. J.*, **43**, 599 (1948).

Experimental

2,5-Diacetoxybenzaldehyde.—The method used was essentially that of Malkin and Nierenstein[3] except that the shaking time was increased to one hour and the product permitted to stand at room temperature for twenty-four hours before filtering and evaporation of the ether. From 13.8 g. (0.1 mole) of 2,5-dihydroxybenzaldehyde[4] one obtains 19.3 g. (87% yield) of crude product, m. p. 66–68°,[5] or 14.2 g. (64% yield) of material in the form of fine white needles, purified by recrystallization from 95% alcohol, m. p. 71–72°.

Anal. Calcd. for $C_{11}H_{10}O_5$: C, 59.46; H, 4.54. Found: C, 59.28; H, 4.89.

The crude material can be used advantageously for the subsequent step of either route. Acetylation in pyridine produced lower yields of purified material.

2-Keto-3-acetamino-6-acetoxycoumarin. (a) **From 2,5-Diacetoxybenzaldehyde.**—An intimate mixture of 21.6 g. of 2,5-diacetoxybenzaldehyde and 18.4 g. of acetylglycine was dried in a vacuum desiccator over calcium chloride for twenty-four hours. After adding 12.8 g. of freshly fused and ground sodium acetate and 60 ml. of 99–100% acetic anhydride the mixture was heated on the steam-bath for forty-five minutes and then permitted to stand at room temperature for twelve hours. The crystalline mass was triturated with 200 ml. of water and permitted to stand for approximately six hours to completely hydrolyze the excess acetic anhydride. The material was filtered, washed with water on the filter and pressed with a dam. The precipitate was placed in a beaker, broken up and suspended in about 50 ml. of ether and again filtered. The product was washed on the filter with about 20 ml. of ether and air-dried. Recrystallization from benzene produced 7.0 g. (27% yield) of purified material as colorless prisms, m. p. 230–231°.

Anal. Calcd. for $C_{13}H_{11}NO_6$: C, 59.77; H, 4.28; N, 5.36. Found: C, 59.79; H, 4.30; N, (Dumas) 5.4, 5.7, 5.5, (Kjeldahl) 5.1, 5.2, 4.9.

Before this method for the preparation of the amino acid was abandoned in favor of the more productive one which made use of benzoylglycine it was thought that this compound was the azlactone. Calcd. for $C_{15}H_{13}NO_6$: C, 59.40; H, 4.33; N, 4.62. Before the six-hour hydrolysis period was used the condensation reaction was treated with ice-water for short periods of time. The precipitate was always oily and difficult to purify but did yield small amounts of material which on purification was obtained as yellow crystals, m. p. 141–142°.[2] It seems probable that the long hydrolysis conducted at room temperature was responsible for the conversion of the true azlactone (m. p. 141–142°) to the acetoxycoumarin (m. p. 230–231°).

(b) **From 2,5-Dihydroxybenzaldehyde.**—The details are the same as those given in (a) above. Thus 13.8 g. (0.1 mole) of 2,5-dihydroxybenzaldehyde, 12.0 g. (0.102 mole)

(3) Malkin and Nierenstein, THIS JOURNAL, **53**, 239 (1931).

(4) Neubauer and Flatow, *Z. physiol. Chem.*, **52**, 380 (1907).

(5) Observed melting points from thermometers calibrated from U. S. P. Melting Point Reference Standards and Anschütz thermometers.

of acetylglycine, 11.2 g. (0.136 mole) of sodium acetate and 44 g. (0.43 mole) of 99–100% acetic anhydride produced 11 g. (38% yield) of the crude acetoxycoumarin, m. p. 180–201°, or 5.4 g. (21% yield) of the purified material, m. p. 230–231°.

2-Phenyl-4-(2,5-diacetoxybenzal)-5-oxazolone. (a) **From 2,5-Diacetoxybenzaldehyde.**—This material had previously been prepared by Neubauer and Flatow[4] from 2,5-dihydroxybenzaldehyde (method b). An intimate mixture of 25.9 g. (0.117 mole) of crude 2,5-diacetoxybenzaldehyde and 19.2 g. (0.216 mole) of benzoylglycine was dried in a vacuum desiccator over calcium chloride for twenty-four hours. One adds 12.0 g. of freshly fused, finely ground sodium acetate and 60 ml. of 99–100% acetic anhydride. The mixture was heated on the steam-bath for forty-five minutes and then during the process of cooling subjected to a partial vacuum which removed approximately 15 ml. of acetic anhydride. After standing at room temperature for a minimum of twelve hours 200 ml. of water was added, the mixture triturated and permitted to stand approximately six hours. The granular material was filtered, resuspended in 100 ml. of water and filtered again and pressed on the filter. The precipitate was suspended in about 50 ml. of ether and filtered, followed by washing on the filter with 25 ml. of ether, and air-dried. This material was light tan in color, weighed 24.1 g. (57% yield based on the crude diacetoxybenzaldehyde or 49% yield based on starting 2,5-dihydroxybenzaldehyde), m. p. 170–187°. When recrystallized from benzene the product melted 195–196°, which is somewhat higher than 190° reported before.[4]

(b) **From 2,5-Dihydroxybenzaldehyde.**—The details are the same as those used in (a) above. After the hydrolysis period the precipitate was of stiff gummy consistency, but after blotting between sheets of filter paper it could be triturated in 50 ml. of ether at which time it became sufficiently granular for successful filtration. Thus 13.8 g. of 2,5-dihydroxybenzaldehyde produced 21.8 g. (60% yield), m. p. 155–172°. When recrystallized from benzene the product weighed 10.4 g. (29% yield), m. p. 195–196°.

2,5-Dihydroxyphenylalanine.—The method used to convert any of the intermediate preparations to the amino acid was based on and made from a combination of methods found in the literature.[6,7,8] The amino acid was prepared by the use of all possible combinations of purified and crude intermediates to find the method which produced the largest amount of purified amino acid from the starting 2,5-dihydroxybenzaldehyde. Twenty-four grams (0.066 mole) of crude 2-phenyl-4-(2,5-diacetoxybenzal)-5-oxazolone (m. p. 170–187° from crude 2,5-diacetoxybenzaldehyde), 120 g. of glacial acetic acid, 120 g. of hydriodic acid (sp. gr. 1.7) and 3 g. of red phosphorus were gently refluxed for ninety minutes. While still hot the mixture was filtered through an asbestos mat and the phosphorus washed with a little hot glacial acetic acid. The filtrate was evaporated to dryness in an atmosphere of hydrogen under diminished pressure. One adds 100 ml. of water and evaporates to dryness as above. The residue was then dissolved as completely as possible in 100 ml. of water at 50° and filtered to remove undissolved benzoic acid. (When prepared from the acetoxycoumarin filtration is unnecessary.) The filtrate was cooled and extracted five times with 40-ml. portions of ether. The water phase was evaporated to approximately 80 ml., a thin layer of ligroin added followed by enough ammonium hydroxide to ensure an excess. The solution was evaporated to dryness as above, and the crude amino acid dissolved in about 150 ml. of hot water containing a little sulfur dioxide. The solution was boiled briefly with a little decolorizing charcoal, filtered and placed in the refrigerator. The amino acid is obtained in a high degree of purity after a single recrystallization from water containing a little sulfur dioxide. The yield of pure material which is obtained in the form of large, colorless crystals containing a molecule of water of crystallization was 7.4 g. (52% yield). The material melts with decomposition between 246 and 254°, depending on the rate of heating.

Anal. Calcd. for $C_9H_{13}NO_5$: C, 50.22; H, 6.09; N, 6.51; H_2O, 8.37. Found: C, 50.20; H, 5.90; N, 6.37; H_2O, 8.68.

The amino acid can be freed of its water of crystallization by drying at 100° for two hours over phosphorus pentoxide at 3 mm. The water was not lost by drying in a desiccator over calcium chloride at room temperature. The amino acid gives a positive ninhydrin reaction, xanthoproteic reaction and ferric chloride solution produces a greenish-black color. Fusion of the material with potassium hydroxide permitted the identification of ammonia. The fusion mass was dissolved in water, acidified with sulfuric acid and extracted with ether. On evaporation of the ether, acetic acid was identified. The *p*-dihydroxyphenyl compound did not survive the treatment with alkali.

When the amino acid was prepared from pure 2-keto-3-acetamino-6-acetoxycoumarin a 63% yield was obtained and from pure 2-phenyl-4-(2,5-diacetoxybenzal)-5-oxazolone the yield was 61%. These yields are equivalent to only 13 and 17%, respectively, when based on starting material 2,5-dihydroxybenzaldehyde.

N-Acetyl-2,5-diacetoxyphenylalanine.—This derivative was prepared because of the general unreliability of the decomposition points of amino acids. Neuberger reports that his product melted at 235° (uncor.). In the purified condition the triacetyl derivative consists of colorless prisms, m. p. 157–158°.

Anal. Calcd. for $C_{15}H_{17}NO_7$: N, 4.33. Found: N, 4.2.

Summary

1. 2,5-Dihydroxyphenylalanine has been prepared from 2-keto-3-acetamino-6-acetoxycoumarin and 2-phenyl-4-(2,5-diacetoxybenzal)-5-oxazolone.

2. The most productive procedure expressed in grams of product and per cent. yields based on starting 2,5-dihydroxybenzaldehyde was: 18.5 g. of 2,5-dihydroxybenzaldehyde yields 25.9 g. of crude 2,5-diacetoxybenzaldehyde (87%); which yields 24.1 g. of crude 2-phenyl-4-(2,5-diacetoxybenzal)-5-oxazolone (49%); which in turn yields 7.4 g. of 2,5-dihydroxyphenylalanine (26%).

ROCHESTER, NEW YORK RECEIVED JUNE 20, 1949

(6) Harington and McCartney, *Biochem. J.*, **21**, 852 (1927).

(7) Lamb and Robson, *ibid.*, **25**, 1231 (1931).

(8) Harington and Randall, *ibid.*, **26**, 1029 (1931).