Synthesis of DL-Tyrosine-4-^{14}C

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SUMMARY

DL-tyrosine-4-^{14}C has been synthesized with an overall yield 29.3 % based on phenol-1-^{14}C as the starting material. Anisole-1-^{14}C was prepared by methylation of phenol-1-^{14}C with dimethyl sulfate. The radioactive anisole was then converted to p-*anisaldehyde-4-^{14}C by a modified Gattermann reaction and then condensed with hydantoin to give 5-(4-methoxybenzylidene-4-^{14}C) hydantoin. The hydantoin was then converted into DL-tyrosine-4-^{14}C.*

INTRODUCTION

Tyrosine as a protein constituent [1], or as a precursor of biologically important substances [2] has been confirmed by using the ^{14}C labelled amino acid, and numerous metabolic pathways of tyrosine have been revealed [3]. Previous studies were perfomed by using single or combination of the following labelled tyrosine : carboxyl-^{14}C-DL-tyrosine [4], DL-tyrosine-α-^{14}C [5], DL-tyrosine-β-^{14}C [6], U-^{14}C-L-tyrosine, and ^{3}H-L-tyrosine [7]. However, none of these five types of labelled compounds are suitable for use *in vivo* and *vitro* studies in which the reaction products are derived from a specific carbon atom of the benzene nucleus.

The carbon atom at position 4 of tyrosine bears a hydroxyl group and has different chemical and physical properties than the 4 position carbon atom of phenylalanine which has no phenolic hydroxyl group.

We were interested in studying certain biological and chemical reactions of tyrosine, in which the carbon atom at the para position would likely be involved. To serve this purpose, the synthesis of DL-tyrosine-4-^{14}C was carried out. The reaction sequences used in the synthesis were as follows :

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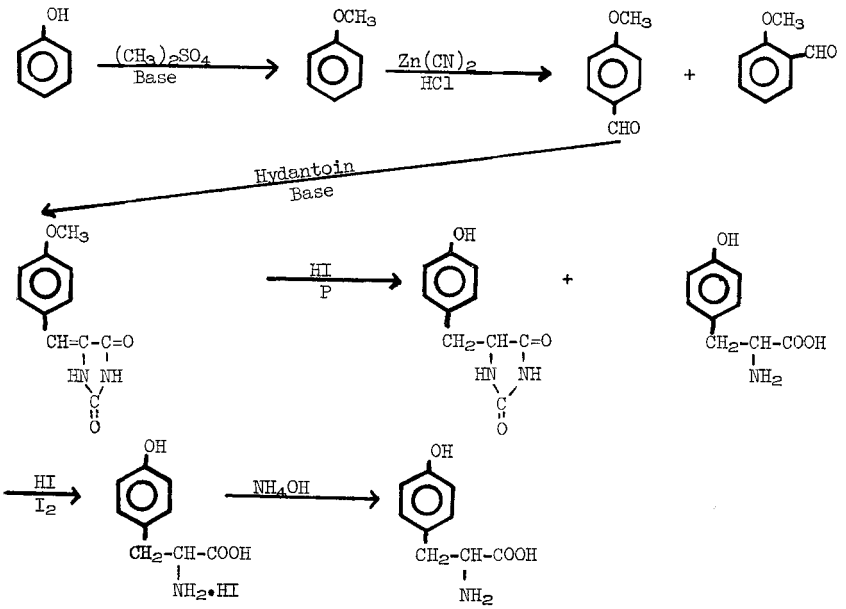

The synthesis of labelled anisole-1-^{14}C has been carried out according to G. S. Hiers and F. D. Hager [8] for the unlabelled compound, but the experimental procedure was modified in order to obtain higher yield. The ratio of the reactants and heating time play an important role in the amount of reaction product.

The conversion of anisole-1-^{14}C to *p*-anisaldehyde-4-^{14}C was attempted by formylation, the Friedel-Craft reaction, Gattermann reaction, or a modified Gattermann reaction [9]. Under ordinary laboratory facilities, the modified Gattermann reaction was superior in yields and procedure, but it was not mentioned whether the reaction product was ortho, para, or a mixture of both forms. R. B. Wagner and H. D. Zook [10] preferred the modified Gattermann synthesis for *p*-anisaldehyde (100 % yield). However, it was found that the synthesized *p*-anisaldehyde-4-^{14}C boiled at 246 °C and consisted of ortho and para isomers which we separated by using thin-layer chromatography.

The condensation reaction of *p*-anisaldehyde-4-^{14}C with hydantoin proceeded smoothly in the presence of a basic catalyst, such as piperidine [11], and the reaction product was subsequently hydrolyzed [12] to DL-tyrosine-4-^{14}C.

Experimental Procedure

Anisole-1-^{14}C.

Initially, 3.5 mg of phenol-1-^{14}C (Volk Chemical Company) was mixed with 4 g of non-radioactive phenol. The specific activity of the mixture was

determined (Packard Tri-Carb Scintillation Spectrometer) after combustion of the phenol to $^{14}CO_2$. The value obtained was 1.42 μc/mmole.

In a 50 ml two-neck round bottom flask equipped with a separatory funnel and a reflux condenser were placed 0.91 g of the mixed phenol, 0.78 g of sodium hydroxide in 7.3 ml of distilled water and 2 or 3 boiling stones. The flask was cooled in an ice bath and 7.4 ml of dimethyl sulfate was introduced slowly into the separatory funnel. The mixture was then heated in a steam bath for thirty minutes. At the end of this time the flask was cooled to room temperature and 0.91 g of the mixed phenol and 0.78 g of sodium hydroxide dissolved in 7.3 ml of distilled water was added. Consequently, a total of 1.82 g of the mixed phenol was used.

The reaction mixture was refluxed for twelve hours and then cooled, and the upper layer which contained the anisole was removed. The aqueous portion was extracted with benzene until repeated extractions gave a negative test to ferric chloride. The benzene extract was combined with the anisole fraction and washed twice using a small amount of distilled water. The trace of water in the anisole-benzene mixture was removed by adding a small amount of anhydrous sodium sulfate. The benzene was removed by distillation over a steam bath, and 1.59 g (81.5 % yield) of the labelled anisole-1-^{14}C was recovered.

p-ANISALDEHYDE-4-^{14}C.

A 50 ml three-neck flask fitted with a reflux condenser, an electric stirrer, and a gas inlet tube was placed in the well-ventilated hood. Into the flask was placed the 1.59 g of anisole-1-^{14}C, 8 ml of dry benzene, and 2.8 g of dry zinc cyanide, which was prepared using zinc chloride and potassium cyanide. A safety bottle was placed between the hydrogenchloride tank and the gas inlet tube. From the top of the condenser a glass tube led to a test tube containing concentrated sulfuric acid. The gas evolved from the reaction flask was bubbled through the sulfuric acid to eliminate water. From the test tube containing the sulfuric acid, a glass tube led to an empty test tube. In a similar manner another glass tube was used to connect the empty test tube to a third tube containing 20 % sodium hydroxide solution. The gas was released in the third test tube near the surface of the basic solution so that the hydrogen cyanide contained in this gas would be removed. With the reaction flask in an ice bath, the mechanical stirrer was started. Dry hydrogen chloride was passed rapidly through the reacting mixture for one hour. Then the supply of gas was stopped, the condenser was disconnected and 2 g of anhydrous aluminium chloride was slowly added into the flask.

After reconnecting the condenser, hydrogen chloride was introduced very slowly while the reaction flask was maintained at 40-45 °C. At the end of four hours the flask was cooled in an ice bath, and ten percent hydrochloric acid was added slowly until the red-brown precipitate turned white. Then this

reaction mixture was refluxed for one hour. An oily portion appeared on the surface of the reaction mixture. The oily fraction was removed, and the aqueous fraction was extracted with benzene until the extraction liquid gave a negative test to 2,4-dinitrophenylhydrazine. Anisaldehyde and the benzene portion were combined, and the traces of water present were removed by using a small amount of anhydrous sodium sulfate. Benzene was then removed on a steam bath, and the residue was the labelled anisaldehyde. The labelled anisaldehyde consisted of both ortho and para isomers. The separation of the two isomers was carried out on thin-layer chromatography plates which were prepared with Silica Gel G using a Stahl Adjustable Applicator, S-11-S. The coated plates (1 mm) were air dried for ten minutes and activated by heating for five hours at 105°-110 °C. The activated plates were then stored in a desiccator until use. For the purpose of separation of ortho and para isomers, 100 μl of the synthetic anisaldehyde were applied to each plate in a strip 10 cm long and 3 cm from the bottom edge. Near each end of the strip a small drop of chemically pure *o*- and *p*-anisaldehyde were placed to serve as reference standards. The spotted plates were developed by the ascending technique using *n*-hexane : ethyl acetate (10 : 1; v/v), which is a modification of the solvent system used by E. Sundt and A. Saccardi [13]. Our solvent system showed distinct separation of these isomers and minimum diffusion of sample due to multiple developments. The development was stopped after the solvent front had reached the top of the plate. The developing solvent adhering to the coating was removed at room temperature, and the dried plates were developed again as described above. This process was repeated four times. After development, the ortho isomer was identified using an ultraviolet lamp (SL-3600, Ultraviolet Products, Inc., San Gabriel, California), as *o*-anisaldehyde is fluorescent and gives a blue color. The para isomer is not visible under ultraviolet light, and its position was determined by spraying the area which contained only the standard *p*-anisaldehyde with hydrazine sulfate. Figure I shows the separation of the *o*- and *p*-anisaldehyde on the plate. Using the spots thus produced, the para isomer was easily identified. The separated *o*- and *p*-anisaldehyde were scraped off from the plate and extracted from the Silica Gel G with ethyl ether. The recovery of standard *o*- and *p*-anisaldehyde from the Silica Gel plate after development was quantitative. The yields of *p*-anisaldehyde-4-^{14}C and *o*-anisaldehyde-2-^{14}C were 1.22 g and 0.79 g, or 60 and 39 % yields, respectively.

5-(4-METHOXYBENZYLIDENE-4-^{14}C)-HYDANTOIN.

Into a round bottom flask, 1.22 g of pure *p*-anisaldehyde-4-^{14}C, 1.3 g of hydantoin, and 1 ml of piperidine were introduced, and the flask was attached to a reflux condenser. The mixture was refluxed at 150°-155° C, for one hour. After cooling, the yellow precipitate was suspended in 100 ml of distilled water. The solution was acidified to Congo Red with 50 % acetic

acid. The supernatant was removed by centrifugation, and the precipitate was washed twice with a minimal amount of hot water. The yellow product was dried in a vacuum desiccator. The yield was 1.50 g (76.2 % of theoretical yield), and the melting point was 244 °C. The mother liquor was evaporated to 10-15 ml using a rotatory evaporator and stored in the cold for three days. A yellow precipitate formed,which was treated as previously described. The yield of the second crop of dried compound was 0.084 g (5.2 %) melting at 240-243 °C. The total yield of 5-(4-methoxybenzylidene-4-^{14}C) hydantoin was 1.584 g (81.4 %).

The same experimental condition for the synthesis of 5-(4-methoxy-benzylidene-4-^{14}C)-hydantoin was employed for the preparation of 5-(2-methoxy-benzylidene-4-^{14}C)-hydantoin. The yield of the latter was 1.51 g (77.7 %), melting point 173 °C.

Infrared absorption spectra of 5-(4-methoxybenzylidene)-hydantoin and 5-(2-methoxybenzylidene)-hydantoin are shown in Figure 2, which were obtained with a Perkin-Elmer infrared spectrometer.

DL-Tyrosine-4-^{14}C.

In a small round-bottom flask were placed 1.584 g of 4-(5-methoxy-benzylidene-4-^{14}C)-hydantoin, 0.6 g of red phosphorus, 7.5 ml of freshly distilled hydriodic acid (specific gravity 1.7), and two glass beads. The flask was refluxed at 190°-200 °C for four hours. The reaction mixture was cooled to room temperature, and 2.15 g of iodine was added. Refluxing was continued for 5.5 hours at 200-210 °C. A clear yellow solution was removed, and the residue was extracted with hot water until the extract showed a negative test to ninhydrin. The extract was added to the yellow solution, and hydriodic acid as well as water were removed by evaporation under reduced pressure. A small amount of distilled water was added to the dried material to remove any remaining hydriodic acid, and the evaporation was repeated. The white solid thus formed was transferred from the evaporating flask to a small beaker using a minimum amount of distilled water and neutralized to pH 7 with concentrated ammonium hydroxide. The neutralized solution was stored in the refrigerator for one day. Tyrosine was filtered and washed twice with distilled water and alcohol. The weight of tyrosine was 0.9304 g (71 %) and melted at 293°-295 °C.

The mother liquor was treated with saturated barium hydroxide solution to remove phosphoric acid until no further formation of a white precipitate occurred. After removing this precipitate by centrifugation, excess Ba^{++} were precipitated by passing carbon dioxide gas through the solution until neutral. The barium carbonate was removed by centrifugation. Traces of Ba^{++} were precipitated as barium sulfate by adding a few drops of concentrated sulfuric acid. The barium sulfate was removed by filtration, and the solution was concentrated using a rotary evaporator and placed in the refrigerator for

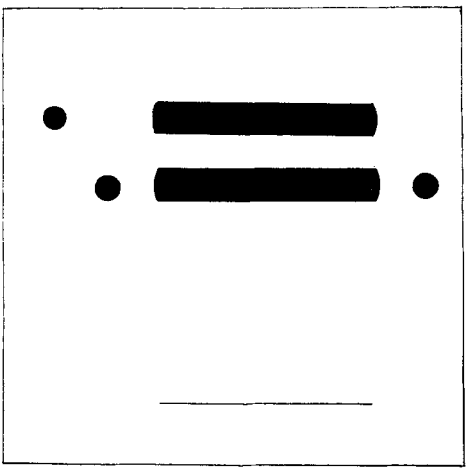

FIG. 1. Separation of synthesized anisaldehyde on TLC for preparatory purposes using multiple development. The plate was developed 5 times using *n*-hexane : ethyl acetate = 10 : 1. Standards of *o*- and *p*- anisaldehyde were spotted on the plate in addition to the band of 100 μl of sample. A : *o*-anisaldehyde, B : *p*-anisaldehyde.

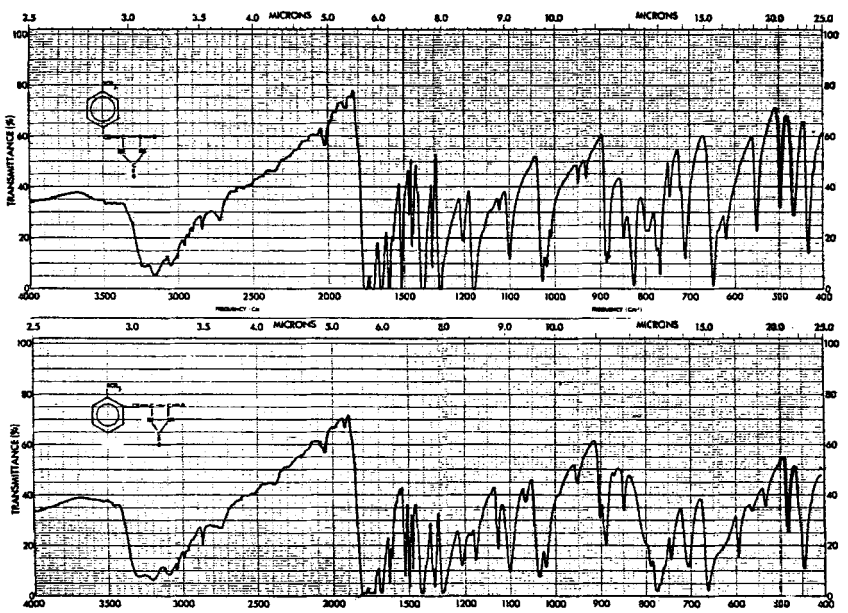

FIG. 2. Comparison of infrared absorption spectra of 5-(4-methoxybenzylidene) hydantoin (upper) and 5-(2-methoxybenzylidene) hydantoin (lower).

two days. During this time a white precipitate of tyrosine appeared. The yield of second crop was 0.023 g (1.73 %), melting point 295 °C.

The total yield of DL-tyrosine-4-^{14}C was 0.95 g (72.73 %), The radioactivity was determined by using a Packard Tri-Carb scintillation spectrometer after combustion of labelled tyrosine to $^{14}CO_2$. The specific activity of synthesized DL-tyrosine-4-^{14}C was 0.248 μc/mmole.

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