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Synthesis and Enzymatic Resolution of DL-2-Phenylglycine-1-^{14}C

Ruth BILLINGS and Hugh R. SULLIVAN
Eli Lilly and Company, Indianapolis, Indiana - 46206
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SUMMARY

DL-2-Phenylglycine-1-^{14}C was prepared in good yield employing a modified Strecker synthesis. The resolution of DL-2-phenylglycine into its pure enantiomorphs was accomplished by the stereoselective enzymatic hydrolysis of the N-chloroacetyl derivative of the L-optical isomer using hog kidney acylase.

In a continuing study of the metabolism of a number of cephalosporin antibiotics it was necessary to prepare 7-(D-α-aminophenylacet [1-^{14}C]amido) cephalosporanic acid. This synthesis and the subsequent metabolic study required pure D-2-phenylglycine-1-^{14}C of relatively high specific radioactivity with the carbon-14 in the 1 position.

The synthesis of the racemic 2-phenylglycine-1-^{14}C was accomplished in good yield employing a modified Strecker cyanohydrin synthesis [1]. The resolution of a racemic amino acid into its enantiomorphic form is normally accomplished employing a chemical technique of fractional crystallization of optically active salts formed by the reaction of the amino acids with an optically active base, i.e., cinchonine. The utilization of this technique, however, for the separation of the enantiomorphs of racemic 2-phenylglycine-1-^{14}C proved disadvantageous because of the small amount of material involved.

A more attractive technique for the resolution of a small quantity of racemic 2-phenylglycine-1-^{14}C was that based upon the stereospecific enzymatic hydrolysis of the N-chloroacetyl derivatives of amino acids employing hog kidney acylase. This general method of resolving amino acids, first described by Greenstein *et al.* [2, 3] is based on the stereospecificity of hog kidney acylase in hydrolyzing only the L enantiomorph of DL-N-chloroacetyl derivatives of amino acids. The D-N-chloroacetyl derivative, after separation of the L-amino acid, is hydrolyzed with dilute acid to yield the D-enantiomorph.

The resolution of DL-2-phenylglycine-1-^{14}C using hog kidney acylase as described by Greenstein *et al.* [4] produced pure enantiomorphs in good yield.

EXPERIMENTAL PART.

DL-2-Phenylglycine-1-^{14}C.

Potassium cyanide-^{14}C, 2,159 g (33.3 m-moles, 12.5 μC/mg available from New England Nuclear Corp., Boston) was placed in a 50 ml round-bottom flask containing a magnetic stirring bar and dissolved in 6.0 ml of water. Ammonium chloride (36.6 m-moles) was added to this solution, and the resulting mixture was stirred at room temperature until complete solution was attained. A solution of 33.3 m-moles of benzaldehyde in 6.0 ml of methanol was added in one portion. Reaction began immediately with the temperature rising to 45° C. The reaction mixture was stirred for 2 hours at room temperature. The reaction solution was diluted by addition of 15 ml of water and was extracted with three 5 ml portions of benzene. The benzene solutions were combined, and the aqueous solution was discarded. The combined benzene solution was washed with three 5 ml portions of water and then extracted with four 10 ml portions of 6*N* hydrochloric acid. The combined acid extract was placed in a 100 ml round-bottom flask and refluxed for 2 hours with stirring. The hydrolysate was cooled and was concentrated *in vacuo* until crystallization of the hydrochloride of DL-2-phenylglycine-1-^{14}C began. The mixture was diluted with 10 ml of water and was treated with 1.0 g of Norit to decolorize and to remove a small amount of resin formed during the hydrolysis. After removal of the Norit by filtration, the filtrate was basified to pH 9.0 with concentrated ammonium hydroxide. Upon cooling, the DL-2-phenylglycine-1-^{14}C was collected by filtration and washed successively with 3.0 ml of water, 5 ml of ethanol and 10 ml of ether. Yield, 1,703 gms (34%).

N-Chloroacetyl-DL-2-Phenylglycine-1-^{14}C.

The DL-2-phenylglycine-1-^{14}C (13.3 m-moles) was placed in a 50 ml round-bottom flask containing a magnetic stirring bar and was dissolved in 6.6 ml of 2*N* sodium hydroxide solution at 5° C. Two additional funnels were placed into the reaction flask, one contained 17.5 m-moles of chloroacetyl chloride and the other 10.0 ml of 2*N* sodium hydroxide. These reagents were added dropwise over a period of 30 min maintaining the pH of the solution at 9.0 and the temperature at 5° C for one hour. The reaction mixture was acidified to pH 2.0 with concentrated hydrochloric acid and chilled to cause crystallization of the N-chloroacetyl-DL-2-phenylglycine-1-^{14}C. The product was collected by filtration and was recrystallized from 7.0 ml of hot water. M.P. 127-8° C. Yield, 1,992 g (65.4%). Reported M.P. 127° C. [5]

Enzymatic Resolution of DL-2-Phenylglycine-1-^{14}C.

The N-chloroacetyl-DL-2-phenylglycine-1-^{14}C, 1,992 g (8.7 m-moles) was dissolved in 90 ml of warm water. The pH of this solution was adjusted to

7.15 with 2*N* lithium hydroxide. Hog kidney acylase, 36 mg, (Nutritional Biochemicals Corp., Cleveland, Ohio, 20,000 μmoles acetyl DL-methionine hydrolyzed/mg protein nitrogen/hr) was added, and the reaction solution was incubated at 37° C (Dubnoff Metabolic Shaking Incubator) for 17 hours. An additional 36 mg of hog kidney acylase was added, and the reaction mixture was incubated for an additional 7 hours at 37° C. The reaction mixture was acidified to pH 5.0 with dilute acetic acid. Norit, 1.0 g, was added with stirring to decolorize the solution and to aid in the removal of protein material. The acid solution was filtered, and the filtrate was lyophilized to yield a white crystalline residue. This residue was dissolved in 6.0 ml of hot water and chilled to facilitate the crystallization of L-2-phenylglycine-1-^{14}C which was collected by filtration. Yield, 520 mg. This material was recrystallized once from water to yield pure L-2-phenylglycine-1-^{14}C, 444 mg (67.5%). $[\alpha]_D^{25} + 148°$ (C = 0.5 in 1*N* hydrochloric acid). Specific radioactivity, 5.83 μC/mg.

The filtrate from the original crystallization was acidified to pH 2.0 with 5*N* hydrochloric acid, whereupon the N-chloroacetyl-D-2-phenylglycine-1-^{14}C crystallized and was collected by filtration, 820 mg (87%). This D-amide was dissolved in 20 ml of 2*N* hydrochloric acid, and the solution was stirred at 100° C for 1 hour. Upon cooling, the acid solution was taken to dryness *in vacuo*; the residue was dissolved in 10 ml of water and again evaporated to dryness *in vacuo*. The white residue was dissolved in 7 ml of water, and the pH of the resulting solution was adjusted to 5.0 with 2*N* lithium hydroxide. After cooling overnight, D-2-phenylglycine-1-^{14}C crystallized from solution, was collected by filtration and was recrystallized from 7 ml of water. Yield 355 mg (54%). $[\alpha]_D^{25} - 148°$ (C = 0.5 in 1*N* hydrochloric acid). Specific radioactivity, 5.50 μC/mg.

REFERENCES

1. Organic Synthesis. — Coll., Vol. III, 84 (1955).
2. Fodor, P. J., Price, V. E. and Greenstein, J. P. — *J. Biol. Chem.*, **178** : 503 (1949).
3. Price, V. E., Gilbert, J. B. and Greenstein, J. P. — *J. Biol. Chem.*, **179** : 1169 (1949).
4. Chemistry of Amino Acids, Greenstein, J. P. and Winitz, M. — Vol. 3, p. 2172 (1961), John Wiley and Son, New York.
5. Dictionary of Organic Compounds. — Heilbron I, Vol. 1, 195 (1965), Oxford University Press, New York.