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Synthesis of DL-Phenylalanine-B-C14

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Synthesis of DL-Phenylalanine- β -C¹⁴

by

James C. Reid

1949 March 1

Berkeley, California

THE SYNTHESIS OF DL-PHENYLALANINE- β -C¹⁴

by

James C. Reid*

Radiation Laboratory and Department of Chemistry,
University of California, Berkeley, California**

ABSTRACT

1 March 1949

The synthesis of DL-phenylalanine- β -C¹⁴ is described. Carboxyl-labeled benzoic acid is reduced to benzylalcohol with lithium aluminum hydride, which after chlorination with SOCl₂ is condensed with acetylaminomalonic ester and the product is hydrolyzed and decarboxylated. The overall yield based on benzoic acid is 40%. Several alternate methods for this preparation are also discussed.

* Present address: National Cancer Institute, National Institutes of Health, Bethesda 14, Maryland.

** The work described in this paper was sponsored by the Atomic Energy Commission.

THE SYNTHESIS OF DL-PHENYLALANINE- β -C¹⁴

by

James C. Reid*

From the Radiation Laboratory,
University of California,
Berkeley, California**

1 March 1949

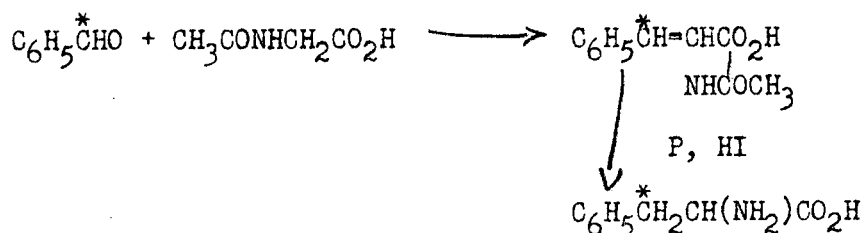
There are three main groups of methods which might be utilized to prepare beta-labeled phenylalanine. Category I encompasses procedures which have in common the condensation of benzaldehyde with various compounds related to glycine, such as acetylglycine (1), hydantoin (2), benzoylthiohydantoin (3), hippuric acid (4), etc., followed by reduction and hydrolysis of the condensation product.

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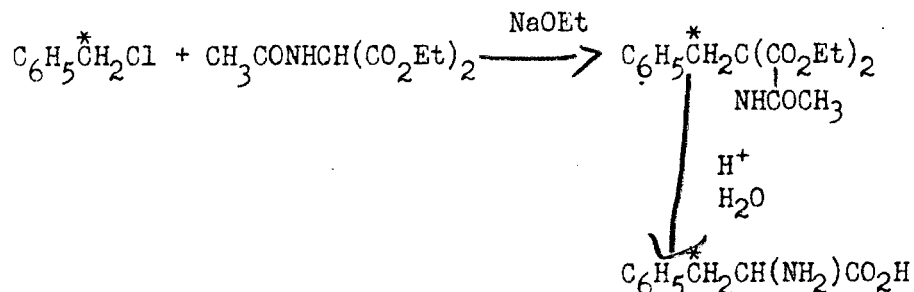
** The work described in this paper was sponsored by the Atomic Energy Commission.

- (1) R. M. Herbst and D. Shemin, *Org. Syntheses Coll.*, Vol. II, 1-3, 489-494 (1939).
 - (2) H. I. Wheeler and C. Hoffman, *Am. Chem. J.*, 45, 368 (1911).
 - (3) T. B. Johnson and W. O'Brien, *J. Biol. Chem.*, 12, 205 (1912).
 - (4) J. Lamb and W. Robson, *Biochem. J.*, 25, 1231 (1931).
-

When benzaldehyde is condensed with acetylglycine for example, the following sequence describes the synthesis (an asterisk is placed on the carbon atom which would be labeled in an active preparation):



Category II includes procedures in which a benzyl halide is condensed with a compound related to aminomalonic acid, such as acetylamino malonic ester (5), phthalimidomalonic ester (6), ethyl acetylamino cyanoacetate (7), etc., followed by hydrolysis to phenylalanine. With acetylamino malonic ester for example, the sequence is as follows:



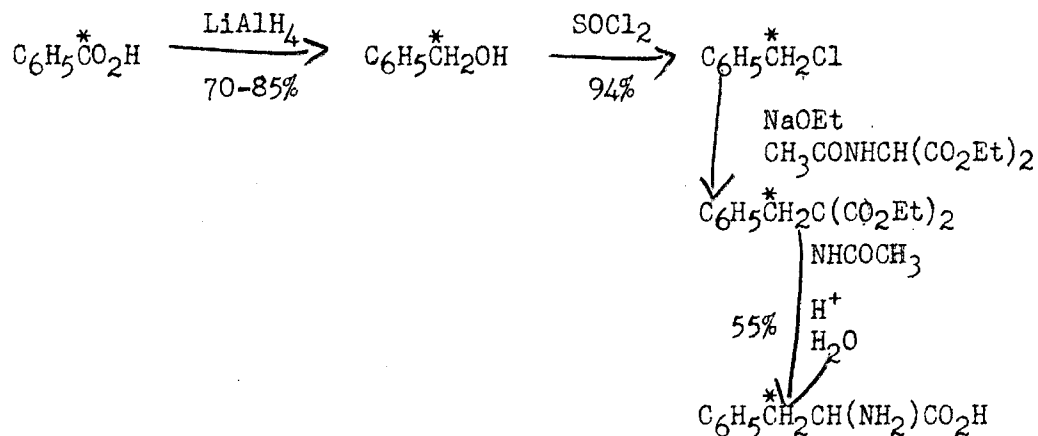
Category III embraces a considerable number of other methods, including Knoop reduction of phenylpyruvic acid (8), reduction of its oxime (9) or phenyl-

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- (5) N. F. Albertson and S. Archer, J. Am. Chem. Soc., 67, 308 (1945).
 (6) S. P. L. Sørensen, Z. Physiol. Chem., 44, 448 (1905).
 (7) N. F. Albertson and B. F. Tullar, J. Am. Chem. Soc., 67, 502 (1945).
 (8) F. Knoop and H. Oesterlin, Z. Physiol. Chem., 148, 311 (1925).
 (9) D. Shemin and F. Herbst, J. Am. Chem. Soc., 60, 195 (1938).
-

hydrazone (10), the Schmidt (11) or the Curtius (12) reaction on benzylcyanoacetic acid or the half ester of benzylmalonic acid, and a few others.

Examination of the literature from the standpoint of the requirements for an economical route for the incorporation of isotopic carbon into the beta position of the alanine side chain suffices to exclude the methods in category III from consideration. A number of experiments were performed with hydantoin, acetylthiohydantoin, and acetylglycine to assess the suitability of the methods in Category I. These experiments showed that the yields in the condensation step do not exceed 70% and the overall yield for the reduction and hydrolysis does not exceed 60%. The indicated yield of labeled benzaldehyde, prepared from isotopic benzoic acid by Rosenmund reduction of benzoyl chloride, is about 70% and the indicated overall yield of phenylalanine prepared by the best method in Category I is accordingly about 29%.

Somewhat better results can be obtained by the methods in Category III. The sequence of reactions finally adopted is as follows:



(10) V. V. Feofilaktov and E. Vinogradova II. Compt. rend. Acad. Sci., U.R.S.S., 24, 759 (1939).

(11) K. F. Schmidt, Ber., 57, 704 (1924).

(12) T. Curtius and A. Sieber, Ber., 55B, 1543 (1922).

EXPERIMENTAL

Benzoic-C¹⁴ Acid. The usual carbonation of phenylmagnesium bromide with isotopic carbon dioxide (13) was used to prepare carboxyl-labeled benzoic acid,

Benzyl-C¹⁴ Alcohol. Carboxyl-labeled benzoic acid was reduced to benzyl alcohol with lithium aluminum hydride (14). A solution of 0.47 g., (12.3 mmoles) of finely powdered lithium aluminum hydride in 35 ml. of dry ether was prepared and filtered through sintered glass. To this solution was added dropwise a solution of 750 mg. (6.14 mmoles) of labeled benzoic acid in 10 ml. of dry ether. A vigorous evolution of hydrogen occurred as each drop fell in and a white precipitate of the lithium aluminum alcoholate accumulated. The addition was made slowly enough to prevent excessive evaporation of ether. All operations were conducted in a dry box under nitrogen. The reaction mixture was allowed to stand overnight in the dry box and excess reagent was decomposed by the cautious dropwise addition of water. Enough hydrochloric acid was then added to dissolve the solids and the benzyl alcohol was extracted with ether. The ether was washed with 5% sodium carbonate solution, which removed a small amount of unconsumed benzoic acid, then dried over anhydrous sodium sulfate and reduced to a volume of 3 ml. The concentrated benzyl alcohol solution was transferred to a short-path still and the benzyl alcohol was recovered by distillation at a pressure of 39 mm. (bath temperature 115°). The yield of colorless product was 481 mg., 71.5%; b.p. (Siwoloboff) 202° at 755 mm. There was

(13) W. G. Dauben, F. E. Yankwich and J. C. Reid, *Anal. Chem.*, 19, 828 (1947).

(14) R. F. Nystrom and W. G. Brown, *J. Am. Chem. Soc.*, 69, 2548 (1947).

some variability in the yield obtained in this step which was apparently connected with variation between different batches of lithium aluminum hydride. Preliminary runs gave yields around 85%.

Benzyl-C¹⁴ Chloride (15). Into a 10 ml. flask was placed a mixture of 481 mg. (4.50 mmoles) of labeled benzyl chloride, 595 mg. (5.00 mmoles) of purified thionyl chloride, 0.01 ml. of dry pyridine and 5 ml. of dry ether. A small reflux condenser was set in place and to its exit was attached a calcium chloride tube. The mixture was refluxed one hour. After being cooled to room temperature the reaction mixture was diluted with 5 ml. of anhydrous ether to precipitate pyridine hydrochloride, centrifuged, concentrated and distilled. Colorless benzyl chloride was collected at pressure of 39 mm. (bath temperature 120°). The yield was 539 mg., 94.4%. A specimen prepared in the same way in a preliminary run with inactive material boiled at 177° (Siwoloboff) and contained 27.86% chlorine. (Theoretical 28.01). The material contained no sulfur.

Condensation of Benzyl-C¹⁴ Chloride with Acetylaminomalonic ester (7). Into a 15 x 150 mm. heavywall Pyrex ignition tube was placed 1.23 g. (5.66 mmoles) of acetylaminomalonic ester and the neck of the tube was drawn to a capillary. Moisture was removed from the interior of the tube by attaching it to a vacuum line, and the tube was transferred to a dry box filled with nitrogen. While the acetylaminomalonic ester was being dried, a solution of sodium ethoxide was prepared in the dry box from sodium and anhydrous ethanol and its concentration determined by titration of an aliquot. Into the ignition tube was placed 7.08 ml. of the

(15) P. Carre and D. Liebermann, Compt. rend., 198, 274 (1934).

solution, containing 5.00 mmoles of sodium ethoxide. The tube was shaken gently and when the acetylamino malonic ester had dissolved a solution of 539 mg. (4.24 mmoles) of radioactive benzyl chloride dissolved in 5 ml. of anhydrous ethanol was added. The tube was capped, removed from the dry box and sealed. The contents were cooled in liquid air before the tube was sealed in order to prevent ignition of ethanol vapor.

The reaction mixture was heated 2-1/2 hours in steam. At the end of this time the tube contained a nearly colorless solution and a white precipitate of sodium chloride. The sodium chloride was removed by centrifugation and the alcoholic solution was evaporated to dryness in a stream of air at room temperature. The residue was dissolved in 5 ml. of hot benzene and centrifuged again to remove a small residue of sodium chloride. Evaporation of the benzene left a white residue of condensation product. The yield was 1.470 g., 113%. (Excess acetylamino malonic ester, which was used to insure complete utilization of labeled benzyl chloride was carried through with the condensation product.)

Hydrolysis of condensation product. The crude condensation product was dissolved in 16.9 ml. of hydrobromic acid (saturated at room temperature) and refluxed under nitrogen 8 hours. A small amount of an oil was formed which was removed by dilution of the solution with 5 ml. of water and extraction with two 5 ml. portions of benzene. The aqueous solution was then evaporated to dryness in high vacuum and dissolved in 5 ml. of water. The solution was charcoaled, filtered and concentrated to incipient crystallization on a steam bath. The hot solution was adjusted to pH 6 with 6 M ammonia solution and the mixture was stored overnight in a refrigerator.

A crop of pure white phenylalanine was collected by filtration, which weighed 437 mg. (55.5%). The overall yield based on benzoic acid was 40%. The product melted at 219-222° (dec.).

Anal.

Found: C, 65.49; H, 6.71; N, 8.79

Calcd. for $C_9H_{11}O_2N$; C, 65.45; H, 6.66; N, 8.48

A substantial amount of phenylalanine remained in the filtrate but could not be recovered uncontaminated by glycine, which arose from hydrolysis of the acetylaminomalonic ester which contaminated the condensation product. This material could of course be obtained in diluted form by the addition of scavenger to the liquor. It was calculated from the solubility of the DL-phenylalanine that the liquor contained about 70 mg. The phenylalanine was prepared with a specific activity of 0.33 microcuries per milligram.