SYNTHESIS OF CARBOXYL-LABELED TRYPTOPHAN FROM HYDANTOIN CONTAINING ISOTOPIC CARBON

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One of the general methods for the preparation of α-amino acids is based on the reaction of the appropriate aldehyde (I) with hydantoin (II), giving the aldehyde hydantoin (III), which is first reduced and then hydrolyzed, giving the corresponding α-amino acid (IV). The preparations by this procedure of phenylalanine (1, 2), tyrosine (1), and tryptophan (3, 4) have been described.

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\begin{align*}
\text{(I)} & : \quad \text{R} - \text{C} - \text{H} + \text{CH}_2 - \text{C} = \text{O} \rightarrow \text{RCH} = \text{C} - \text{C} = \text{O} \\
\text{(II)} & : \quad \text{HN}^1 - \text{C} - \text{NH}^2 - \text{C} = \text{O} \\
\text{(III)} & : \quad \text{HN}^1 - \text{C} - \text{NH}^2 - \text{C} = \text{O} \\
\text{(IV)} & : \quad \text{RCH}_2 - \text{C} - \text{CH}_2 - \text{C} = \text{O} \quad \text{Hydrolysis} \rightarrow \text{RCH}_2 - \text{C} - \text{CH}_2 - \text{C} = \text{O} \\
\end{align*}
\]

The use of hydantoin labeled with isotopic carbon in position 4 in the synthesis would lead to the corresponding carboxyl-labeled amino acid. This has been demonstrated by the preparation of labeled hydantoin and its conversion to carboxyl-labeled tryptophan.

Hydantoin-4-C\textsuperscript{13} was prepared in four steps from isotopic potassium cyanide according to the scheme, potassium cyanide \(\rightarrow\) glycine (V) \(\rightarrow\) glycine ethyl ester hydrochloride (VI) \(\rightarrow\) hydantoic ethyl ester (VII) \(\rightarrow\) hydantoin (VIII).
SYNTHESIS OF TRYPTOPHAN

The yield of hydantoin based on potassium cyanide was 57 per cent. The yield of tryptophan based on hydantoin was 59 per cent, giving an over-all yield from potassium cyanide of 33 per cent.

EXPERIMENTAL

Synthesis of Hydantoin Containing Isotopic Carbon

Isotopic Glycine—The directions of Sakami, Evans, and Gurin (5) were followed exactly. From 2.68 gm. of isotopic potassium cyanide which analysis showed to contain 20.7 per cent excess C13, 1.93 gm. (62.5 per cent) of glycine, m.p. 236-237° (decomposition), was obtained. An authentic sample of glycine melted at 235° (decomposition).

Isotopic Glycine Ethyl Ester Hydrochloride—The directions of Harries and Weiss (6) were followed. The labeled glycine was covered with 10 ml. of absolute ethanol and dry hydrogen chloride was bubbled through the mixture while suspended in a 65-70° oil bath. The glycine slowly dissolved during the course of 30 minutes. Alcohol was added to replace that which evaporated. Treatment with hydrogen chloride was continued for an additional 15 minutes. In practice runs the ester hydrochloride would occasionally crystallize during this last period. The mixture was cautiously evaporated to dryness on a warm surface, giving 3.49 gm. (98.0 per cent) of glycine ethyl ester hydrochloride, m.p. 145.2°.

Isotope Analysis—Excess C13. Calculated, 5.18; found, 5.16, 5.08

1 All Analyses were performed on the consolidated mass spectrometer of the National Bureau of Standards. I wish to thank Dr. Fred L. Mohler and his staff for these analyses.

2 All melting points were taken with the same apparatus, except where noted, and are uncorrected.
Isotopic Hydantoic Ethyl Ester—The directions of Harries and Weiss were followed. The labeled glycine ethyl ester hydrochloride was dissolved in 4 ml. of water, and to it was added a slurry of 3.0 gm. of freshly prepared potassium cyanate (7) in 7.0 ml. of water. The mixture was stirred until precipitation started (4 to 5 minutes), and was then cooled at -5° for 2 hours, filtered, and dried, giving 3.40 gm. (92.4 per cent) of hydantoic ethyl ester, m.p. 129.3-129.8°.

Isotope Analysis—Excess C. Calculated, 4.14; found, 4.21, 4.31

Isotopic Hydantoin—The directions of Harries and Weiss and of Wagner and Simons (8) were followed. The labeled hydantoic ethyl ester was covered with 15 ml. of 25 per cent hydrochloric acid and evaporated to dryness on a steam bath overnight, giving 2.67 gm. of crude hydantoin. This product was obviously impure, since the yield exceeded the theoretical (2.33 gm.) and the melting point, 203-206°, was low. Fortunately, however, it was found in trial runs that this crude material could be used directly for the synthesis of indolylidenehydantoin without recourse to purification by alcohol extraction such as that recommended by Wagner and Simons.

Since in trial runs in which purification was effected, the yield of hydantoin regularly approached quantitative amounts, such a yield is assumed here, giving an over-all yield of hydantoin based on potassium cyanide of 57 per cent.

Isotope Analysis—Excess C. Calculated, 6.91; found, 6.86, 6.97

Synthesis of Carboxyl-Labeled Tryptophan

Isotopic Indolylidenehydantoin—The directions of Shabica et al. (9) were followed. The labeled hydantoin was mixed with 3.38 gm. of indole-3-aldehyde and 9.0 ml. of piperidine in a 109 ml. flask equipped with a reflux condenser. The flask was lowered into an oil bath maintained at 150° for 20 minutes. The canary-yellow indolylidenehydantoin which formed was suspended in 250 ml. of water, acidified to Congo red paper with acetic acid, filtered, and washed with water, giving 5.00 gm. (94.5 per cent) of indolylidenehydantoin, m.p. 314-314.5° (metal block); authentic sample, m.p. 314°.

Isotope Analysis—Excess C. Calculated, 1.73; found, 1.50, 1.62

Isotopic Indolylmethylhydantoin—The directions of Elks et al. (10) were followed exactly. It was found in practice that the Raney nickel used had to be quite active; otherwise the period of hydrogenation was unduly prolonged from the normal 6 hours to about 24, and the resulting product melted over a wide range after softening at 218°. Fortunately, however, this product gave tryptophan upon hydrolysis. It was presumed that
partial hydrolysis occurred during the long contact with sodium hydroxide. The yield of indolylmethylhydantoin was 3.57 gm. (71.0 per cent), m.p. 218°. The yield in practice runs was usually about 90 per cent.

Isotope Analysis—Excess C\textsubscript{13}. Calculated, 1.73; found, 1.75, 1.76

Isotopic Tryptophan—The directions of Elks et al. were followed except for two modifications: the indolylmethylhydantoin was hydrolyzed for 40 instead of 24 hours, and the final product was evaporated to dryness on a steam bath instead of \textit{in vacuo}. Yield, 2.80 gm. (88.0 per cent), m.p. 268°.

Isotope Analysis—Excess C\textsubscript{13}. Calculated, 1.88; found, 1.93, 1.98

The isotopic tryptophan and unlabeled tryptophan made by the same process in practice runs showed full tryptophan activity when tested by biological assay.\textsuperscript{3}

The author is indebted to Mr. Clarence E. Emery, Jr., for technical assistance in carrying out many of the practice runs.

\textbf{SUMMARY}

Hydantoin labeled with isotopic carbon has been prepared from isotopic potassium cyanide in 57 per cent yield.

Carboxyl-labeled \textit{dl}-tryptophan has been prepared from the labeled hydantoin in 59 per cent yield.

\textbf{BIBLIOGRAPHY}


\textsuperscript{3} Analysis by Dr. James M. Hundley of this Institute.
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