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The Synthesis of DL-Tryptophan-B-C14, INDOL-3-Acetic Acid-a-C14 and DL-Tryptophan-3-C14

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THE SYNTHESIS OF DL-TRYPTOPHAN-β-C$^{14}$, INDOLE-3-ACETIC ACID-α-C$^{14}$
AND DL-TRYPTOPHAN-3-C$^{14}$

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March 15, 1949

Berkeley, California
THE SYNTHESIS OF DL-TRYPTOPHAN-β-C¹⁴, INDOLE-3-ACETIC ACID-α-C¹⁴
AND DL-TRYPTOPHAN-3-C¹⁴*

by

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March 15, 1949

ABSTRACT

DL-tryptophan-β-C¹⁴, indole-3-acetic acid-α-C¹⁴ and DL-tryptophan-
3-C¹⁴ have been synthesized.

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* This system of nomenclature is in accord with that proposed by Calvin, Heidelberger, Reid, Tolbert and Yankwich in "Isotopic Carbon," John Wiley and Sons, Inc., (1949). The β-carbon is in the side-chain and the 3-carbon is in the indole ring.

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THE SYNTHESIS OF DL-TRYPTOPHAN-β-C\textsuperscript{14}, INDOLE-3-ACETIC ACID-α-C\textsuperscript{14} 
AND DL-TRYPTOPHAN-3-C\textsuperscript{14} *

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The first recorded synthesis of tryptophan by Ellinger and Flamand in 1908 (1) involved the condensation of indole-3-aldehyde and hippuric acid in a typical azlactone reaction. The yield in the synthesis was much improved in 1935 by Boyd and Robson (2) who employed hydantoin instead of hippuric acid for the condensation. This reaction has recently been used by Bond (3) in the preparation of DL-tryptophan-carboxyl-C\textsuperscript{14}. In 1944

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(3) H. W. Bond, J. Biol. Chem., 175, 531 (1948).
Snyder and Smith (4) and Albertson, Archer and Suter (5) independently reported almost identical syntheses of tryptophan by the condensation of gramine with ethylacetamidomalonate followed by hydrolysis. Suitable modifications of these procedures were employed in the synthesis of DL-tryptophan-β-Cl. The isotopic indole acetic acid was prepared from gramine by modifications of a known method (6) which involves alkylation of potassium cyanide by the gramine, followed by hydrolysis. The reactions and the yields obtained are indicated by the equations.

\[ \text{H} \text{N} \quad \text{CH}_2 \text{O} + (\text{CH}_3)_2 \text{NH} \xrightarrow{\text{HAc}} \text{H} \text{N} \text{CH}_2 \text{N}(\text{CH}_3)_2 \]

33% based on CO₂

\[ \begin{align*}
\text{HCOOH} & \xrightarrow{\text{NaOH}} \text{H}_2\text{O} \text{(COOEt)}_2 \\
\text{HCOOCH}_3 & \xrightarrow{\text{Heat}} \text{H} \text{N} \text{CH}_2 \text{N}(\text{COOEt})_2 \\
\text{H} \text{N} & \xrightarrow{\text{H}_2\text{SO}_4} \text{H}_2\text{CHOOH} \\
\text{NH}_2 & \xrightarrow{\text{NH}_2} \text{H}_2\text{CHOOH}
\end{align*} \]

76%

The isotopic formaldehyde was prepared from methanol which was obtained from carbon dioxide by high pressure hydrogenation (7). The preparation of DL-tryptophan-3-C¹⁴ is indicated in the Experimental section.

EXPERIMENTAL

The formaldehyde was prepared as described in "Isotopic Carbon." (8)

Copper hydroxide, freshly precipitated from a cupric nitrate solution by the addition of dilute ammonium hydroxide, was washed thoroughly with water and applied to a copper screen, which was rolled, inserted into a small quartz tube and reduced with hydrogen at 450°. The labeled methanol, which contained water, was passed over the catalyst (600°) with a stream of air from a small bubbler heated to 70°. The reduction products were trapped in a small bubbler containing 1 ml. of water and a trace of methanol. The unabsorbed gases were passed through a combustion furnace to recover the radioactive carbon. The yields of formaldehyde varied from 40-60%. The formaldehyde, thus obtained from methanol following the reduction of 25 mmoles of labeled carbon dioxide, was added to a chilled mixture of 1.42 g. of 33% aqueous dimethylamine and 1.42 g. of glacial acetic acid and the solution was quickly added to 1.10 g. of indole. Heat was evolved, the indole dissolved and the mixture was allowed to stand at room temperature for 18 hours. The light yellow solution was then added dropwise to an ice-cold solution of 1.42 g. of sodium hydroxide in 20 ml. of water and a white crystalline precipitate of gramine was formed which melted at 121-126°. Yield, 1.39 g. (33% based on carbon dioxide). Specific activity 152,000 cts./min./mg.

The crude gramine, 1.29 g., was allowed to react at room temperature with a mixture from 0.18 g. of sodium in specially dried absolute ethanol, 1.70 g. of ethyl acetamidomalonate and 1.2 ml. of dimethyl sulfate was added as described (5). Yield, 2.10 g. (82%), m.p. 131-133.5°.


* Mallinkrodt Chemical Co. reagent grade copper nitrate gave better yields of formaldehyde than any of five other samples tested.
The ester, 2.10 g., was refluxed for three hours with a solution of 1.20 g. of sodium hydroxide in 10 ml. of water and on cooling and acidification 1.57 g. (90%) of the malonic acid, m.p. 135-138° (dec.) was obtained. This compound was heated to 140°, smooth decarboxylation occurred and the acetyl tryptophan so obtained was recrystallized from an alcohol-water mixture containing a trace of sodium hydrosulfite to give a yield of 92%, m.p. 206-208°. Hydrolysis was accomplished by heating 0.53 g. for four hours under nitrogen with 6 ml. of 2 N sulfuric acid. About 30 ml. of water was added and the solution was heated, decolorized with charcoal and neutralized to phenolphthalein with barium hydroxide. The hot solution was filtered free of barium sulfate and was evaporated to dryness. The resulting tryptophan was recrystallized almost quantitatively from 15 ml. of acetic acid and 15 ml. of benzene, and 0.54 g. (82%) was obtained of glistening, colorless plates of tryptophan acetate salt containing a molecule of acetic acid of crystallization. This represents an overall yield of 19% based on carbon dioxide. Specific activity, 132,000 cts./min./mg. (approximately 1 µc/mg.) Analysis: Calc'd for C_{15}H_{20}O_{6}N_{2}: C, 55.52; H, 6.15. Found: C, 55.52; H, 6.12.

Indole-3-Acetic Acid-$\alpha$-$^{14}C$: Gramine, 50 mg., and potassium cyanide, 38 mg., were dissolved in 1 ml. of 50% ethanol, 0.1 ml. of dimethyl sulfate was added and the mixture was refluxed for 1-1/2 hours. The alcohol was then distilled and the crude nitrile separated as a yellowish oil which was extracted with ether. After evaporation of the ether, the nitrile was refluxed for 48 hours with 50 mg. of potassium hydroxide dissolved in 2.5 ml. of ethanol with 10 drops of water. The alcohol was then evaporated and more water was added. The solution was extracted with ether to re-
move a small amount of nitrile and was then acidified. The indole acetic acid thus obtained was recrystallized from water. Yield, 25 mg. (50%, m.p. 163-165°). Specific activity, 130,000 cts./min./mg. Analysis: Calcd for C\(_{10}\)H\(_9\)O\(_2\)N - C, 68.56; H, 5.18. Found: C, 68.32; H, 5.29. The acid was then converted to the sodium salt for storage.

**DL-Tryptophan-3-C\(^{14}\):** This ring-labeled tryptophan was prepared in very low yield by the following series of reactions in which no special modifications were made in the standardized procedures:

\[
\begin{align*}
C_6H_5^*COOH & \xrightarrow{94\%} C_6H_5^*COOC_2H_5 & \xrightarrow{73\%} C_6H_5^*CH_3 & \xrightarrow{58\%}
\end{align*}
\]

\(9\%\) and \(p\)-nitrotoluenes.

\(8\%\) and \(p\)-aminotoluenes \(\rightarrow\) \(9\%\) and \(p\)-formylaminotoluenes \(\rightarrow\)

Indole-3-C\(^{14}\) Tryptophan-3-C\(^{14}\) as before.

Carboxyl-labeled benzoic acid, 10 g., was esterified by the azeotropic method (9), and the ethyl benzoate was hydrogenated to toluene at 250° over copper chromite catalyst (10). The toluene was nitrated in sulfuric acid solution (11), and the mixture of \(9\%\) and \(p\)-nitrotoluene was hydrogenated in benzene solution at 210° over a nickel on kieselguhr catalyst (12). The crude amines were refluxed with 90% formic acid, and converted into indole (0.39 g.) by treatment with potassium tert-butoxide.

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followed by pyrolysis (13). Only the ortho isomer gave indole. The overall yield of indole from benzoic acid was 8.5%. The indole was then converted into tryptophan, using non-isotopic formaldehyde, by the series of reactions used for the β-labeled compound and in substantially the same yield. Specific activity, 6800 cts./min./mg.

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SUMMARY

DL-tryptophan-β-\( ^{14} \)C, indole-3-acetic acid-\( \alpha-^{14} \)C and DL-tryptophan-\( 3-^{14} \)C have been synthesized.