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THE SYNTHESIS OF LABELED ALPHA AMINO ACIDS

James C. Reid and B. M. Tolbert

August 1, 1949

Berkeley, California

THE SYNTHESIS OF LABELED ALPHA AMINO ACIDS*

A Review of Published Work Done Up to April 1, 1948

by

James C. Reid and B. M. Tolbert

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

ABSTRACT

August 1, 1949

The literature on the synthesis of isotopically labeled amino acids (both active and inactive isotopes) has been reviewed, and a short, comprehensive outline of the chemicals used is presented.

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*. To be included as a chapter in a forthcoming book on amino acids edited by D. M. Greenberg.

** This paper was written under the auspices of the Atomic Energy Commission.

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I.

INTRODUCTION

Among the newer investigational methods available to the biochemist is the use of rare or artificial isotopes as tracers. The basis of the method is the fact that two isotopes of an element are so nearly identical in their chemical properties that in biological work the differences are not very important. As an example, glycine ($H_2NCH_2CO_2H$) can be prepared in such a way that the carboxyl group contains carbon whose content of the rare isotope C^{13} is greater than normal. Then the metabolic fate of the carboxyl carbon atoms of glycine when administered to a living organism can be followed by ascertaining in which of the compounds present in the organism an abnormally high concentration of C^{13} appears. The finding of extra C^{13} in a compound means that the organism synthesizes the compound by a method which utilizes, directly or

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indirectly, the carboxyl group of glycine. If glycine is prepared with the alpha-carbon position labeled, the fate of this part of the molecule can be learned, and by using a rare isotope of nitrogen, the amino group can be followed.

A rare hydrogen isotope, too, can be used to label the glycine. Although this may be done in certain cases in order to follow the hydrogen, the purpose is more commonly to use the hydrogen as a tracer for the carbon to which it is attached. Thus, glycine in which the hydrogen attached to the alpha-carbon atom is enriched in deuterium can be administered to an animal and then located in the tissues by means of deuterium analysis, provided, of course, that the deuterium has not become separated from the carbon. This requirement, that the hydrogen be stably bound to the atom for which it serves as a tracer, places certain limitations upon its use in labeling compounds. It is not possible to use hydrogen as a tracer for the amino group of glycine, for instance, because in the body of an experimental animal the deuterium of the amino group will exchange with the hydrogen of water and the amino hydrogen will rapidly approach the isotopic composition of ordinary hydrogen. The amino group of the glycine will then no longer be distinguishable from any other amino group.

Tracer isotopes may be of either the stable or radioactive variety. The concentration of a stable tracer in a specimen under observation is determined by a method of measuring mass differences, such as density measurement or mass spectrometer assay; an unstable tracer is determined with a Geiger-Müeller counter or other device for measuring radioactivity.

Satisfactory sources of tracer isotopes are available for all the elements of major biochemical importance with the exception of oxygen. It is

experiment requires that a particular position in a molecule be labeled rather than any other position, the choice of synthetic method will be further narrowed. Thus, a method satisfactory for preparing glycine labeled with carbon in the carbonyl group is not necessarily suitable for preparing glycine labeled at the alpha carbon atom. (4) With certain radioactive isotopes, the amount of activity necessary to conduct a series of experiments within the activity limits required by tracer experiments may be contained in only a few hundred milligrams of product; thus, the synthetic methods should be adaptable to operation with small quantities of material (a gram or less). (5) The health hazard presented by radioactive substances limits the amount of activity which can be prudently handled in an ordinary laboratory.

II.

INTRODUCTION OF DEUTERIUM AND TRITIUM (D_2 AND T_2) INTO AMINO ACIDS

The synthesis of amino acids labeled with isotopic hydrogen is accomplished by methods which involve either the direct exchange of normal for isotopic hydrogen or the reduction of an unsaturated linkage with isotopic hydrogen.

Exchange Reaction: - The exchange reaction has the advantage that by its use many compounds which are difficult to synthesize can be easily labeled. The conditions necessary for exchange vary with the compound; in general, hydrogen attached to an aromatic carbon atom exchanges more readily than when bound to an aliphatic one, and the lability is increased by the presence of an activator, such as an ortho hydroxyl group. On the other hand, the rate of exchange of hydrogen bound to an aliphatic carbon atom can be increased by the presence of an acid strengthening group such as an α-unsaturation. By vary-

ing the experimental conditions a variety of compounds can be labeled with isotopic hydrogen. Optically active amino acids are racemized when the alpha hydrogen is replaced.

The following amino acids have been prepared by the exchange of hydrogen with strong (80-90%) deuterio- or tritio-sulfuric acid: deuterio-DL-phenylalanine (1), deuterio-DL-alanine (2,3), deuterio-DL-Leucine (2,3), and tritio-DL-phenylalanine (4).

Isotopic hydrogen has also been introduced into amino acids by the use of more dilute acid (20% hydrogen chloride or sulfuric acid in heavy water) by prolonged heating at elevated temperature. Isotopes introduced in this way can be removed under the same conditions by reverse exchange if the solvent is ordinary water. This consideration places a limitation on the use of amino acids labeled with dilute acid in experiments where they will be subjected to the action of hot acid (as in the hydrolysis of a protein). Concentrated sulfuric acid, on the other hand, is capable of causing exchange of hydrogen too stably bound to be removed by heating with 20% acid and compounds labeled in this way can be used with less danger of loss of tracer caused by subsequent chemical manipulation. By the use of dilute acid in heavy water the following compounds have been prepared: deuterio-glycine (3), deuterio-L-proline (3), deuterio-cystine (3,5), deuterio-tyrosine (3), deuterio-L-glutamic acid (3), deuterio-arginine (5) and deuterio-lysine (5). After several days' heating, the extent of exchange is small in all cases.

In the presence of platinum black many compounds will exchange hydrogen with heavy water. Amino acids themselves do not exchange well, but the method can be used to prepare labeled starting materials from which amino acids may

be synthesized. Thus, deuterio-isocaproic acid has been made by exchange and used to prepare deuterio-DL-leucine (6):

$$(CH_3)_2CHCH_2CH_2CO_2H \xrightarrow[Pt]{D_2O} (\overset{*}{C}H_3)_2C\overset{*}{H}\overset{*}{C}H_2\overset{*}{C}H_2CO_2\overset{*}{H} \xrightarrow{P,\ Br_2}$$

$$(\overset{*}{C}H_3)_2\overset{*}{C}H\overset{*}{C}H_2\overset{*}{C}HBrCO_2\overset{*}{H} \xrightarrow[2.\ \text{Hydrolysis}]{1.\ \text{K phthalimide}} (\overset{*}{C}H_3)_2\overset{*}{C}H\overset{*}{C}H_2\overset{*}{C}H(NH_2)CO_2H$$

Yield, 44-53% based on $\overset{*}{O}_2$ (a)

(a) The asterisk is used to designate the location of the tracer isotope

Leucine labeled with both deuterium and N^{15} has been prepared by the same reaction sequence using potassium phthalimide-N^{15} (Section III). By the same sequence of reactions deuterio-DL-valine has been prepared from labeled isovaleric acid (6).

Reduction of an Unsaturated Linkage: - The hydrogenation of an unsaturated linkage as a method of introducing isotopic hydrogen has the advantage that isotope introduced in this way is ordinarily stably bound.

A particularly convenient reduction procedure is the Knoop reaction, in which an α-keto acid is converted to an amino acid by treatment with hydrogen and aqueous ammonia in the presence of a platinum or palladium catalyst. By substituting isotopic for ordinary hydrogen in the procedure, DL-glutamic acid-α,β-d can be prepared (7) from α-ketoglutaric acid:

$$HO_2CCH_2CH_2COCO_2H \xrightarrow[Pt]{D_2 + NH_3} HO_2CCH_2\overset{*}{C}H_2\overset{*}{C}H(NH_2)COOH$$

A certain amount of isotope can also be introduced into the glutamic acid if heavy water is used as the solvent and ordinary hydrogen is used for

the reduction. This procedure gives a different distribution of isotope in the molecule. The Knoop reaction has also been used to prepare glutamic acid labeled with both deuterium and N^{15} (Section III).

Deuterio-DL-alanine has been prepared (8) by the Knoop reaction, with gaseous deuterium.

Deuterio-methanol has been prepared by catalytic reduction of carbon monoxide and converted to deuterio-methyl iodide. The methyl iodide was then used to prepare labeled methionine (9):

$$CO \xrightarrow{D_2} \overset{*}{C}H_3\overset{*}{O}H \xrightarrow{P,\ I_2} \overset{*}{C}H_3I \xrightarrow[\text{in liquid } NH_3]{\text{Homocystine + Na}} \overset{*}{C}H_2SCH_2CH_2CH(NH_2)CO_2H$$

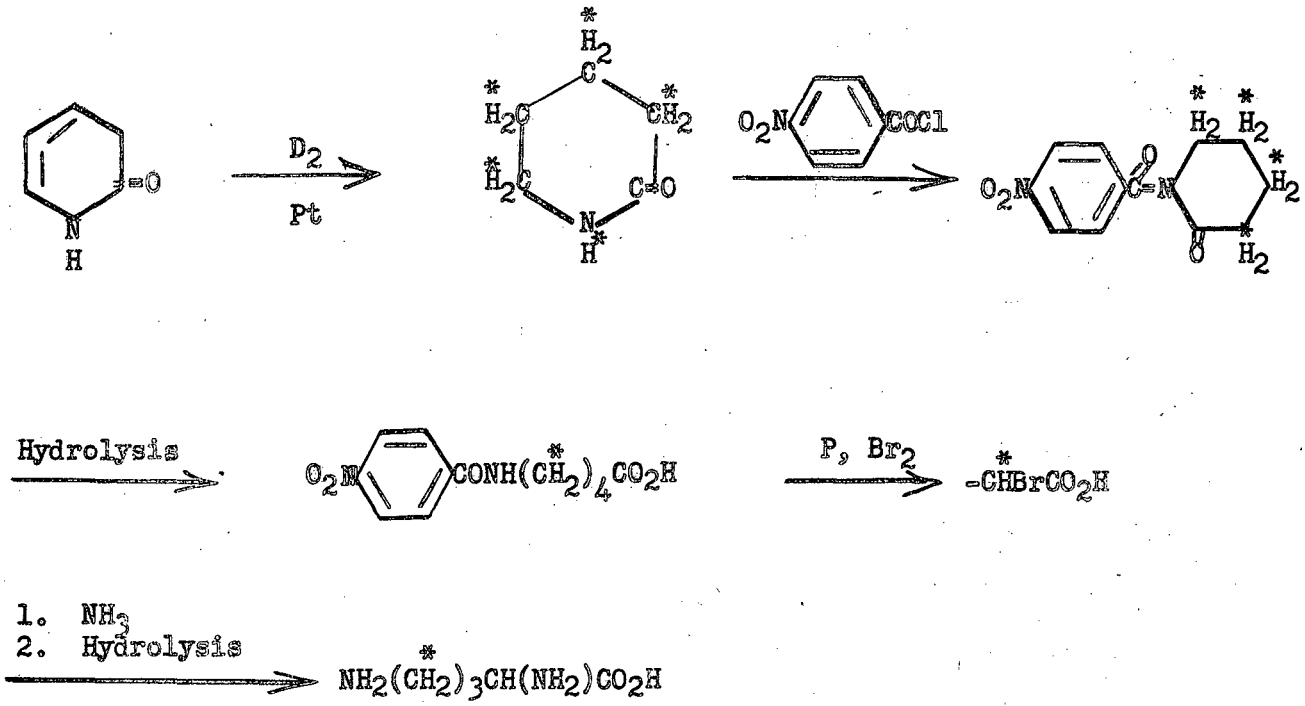
Reduction of an unsaturated acetal provides a route to a labeled aldehyde, which can then be converted to an amino acid. In this way DL-leucine-β,γ-d has been prepared (10) from isopentenal diethyl acetal:

$$(CH_3)_2C{=}CHOH(OEt)_2 \xrightarrow{D_2} (CH_3)_2\overset{*}{C}H\overset{*}{C}H_2CH(OEt)_2 \xrightarrow[H^+]{H_2O}$$

$$(CH_3)_2\overset{*}{C}H\overset{*}{C}H_2CHO \xrightarrow[KCN]{NH_4Cl} (CH_3)_2\overset{*}{C}H\overset{*}{C}H_2CH(NH_2)CO_2H$$

In the same way, DL-valine-β,γ-d has been prepared (10) from isobutenal diethyl acetal.

Catalytic reduction has been employed to convert α-pyridone to α-piperidone which was then used to prepare DL-ornithine-α,β,γ,δ-d (11):



Labeled α-pyridone has also been used to prepare proline labeled with both deuterium and N^{15} (see Section III - amino acids labeled with N^{15}).

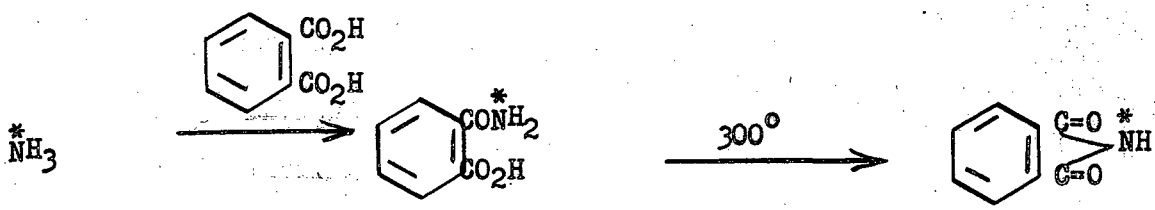
Deuterio-cyclohexanone prepared by the catalytic reduction of phenol can be used as a starting point for the preparation of doubly labeled lysine (Section III). Attempts to prepare deuterio-cyclohexanone by direct exchange between cyclohexanone and deuterium oxide in the presence of platinum fail to give a satisfactory yield.

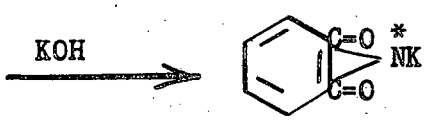
III.

INTRODUCTION OF N^{15} INTO AMINO ACIDS

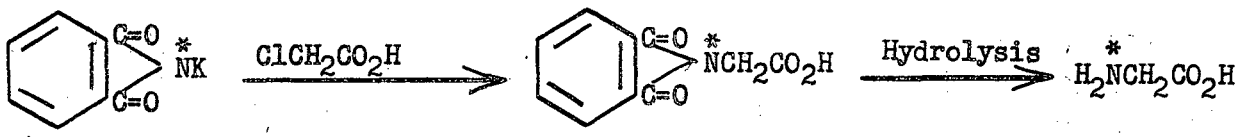
In general, amino acids do not exchange their α-amino nitrogen with other nitrogenous compounds under ordinary conditions (14), and exchange reactions cannot be used to introduce the tracer. Neither is the classical reaction between an α-halo acid and ammonia a convenient route, because a large excess of ammonia is required, a feature which makes for inefficient utilization of the enriched starting material. Several synthetic methods have been successfully adapted to tracer work, however; of these the two most generally applicable are the Gabriel and the Knoop syntheses. The Strecker reaction also finds application.

Gabriel Synthesis: - Isotopic ammonia can be converted to potassium phthalimide, practically without loss (13):



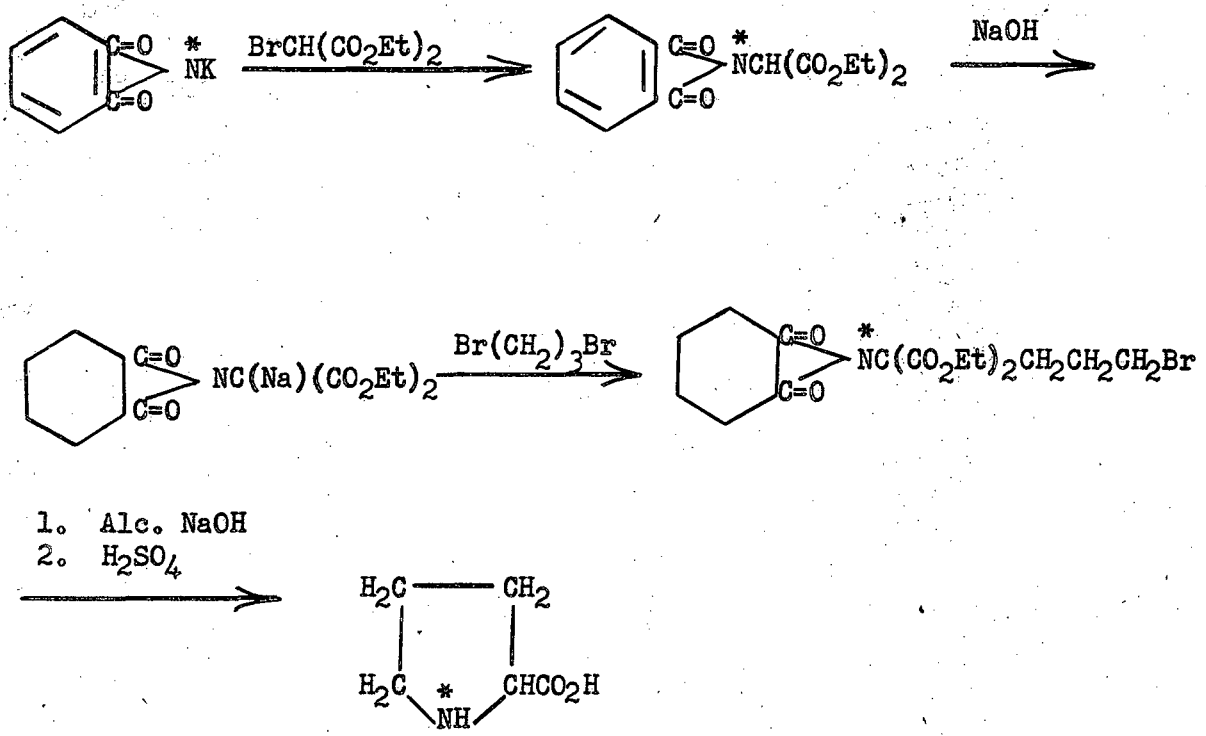


The labeled phthalimide can then be converted to a variety of amino acids by condensation with the proper α-halo acids. Thus, glycine-N^{15} (13,15,16) has been prepared from chloroacetic acid in 89% yield, based on potassium phthalimide:



By this use of the Gabriel synthesis DL-lysine-N^{15} (13) in 72% yield, based on phthalimide and DL-serine-N^{15} (15,17) have also been prepared. D-serine-N^{15} and L-serine-N^{15} were prepared (15) from the synthetic racemic mixture.

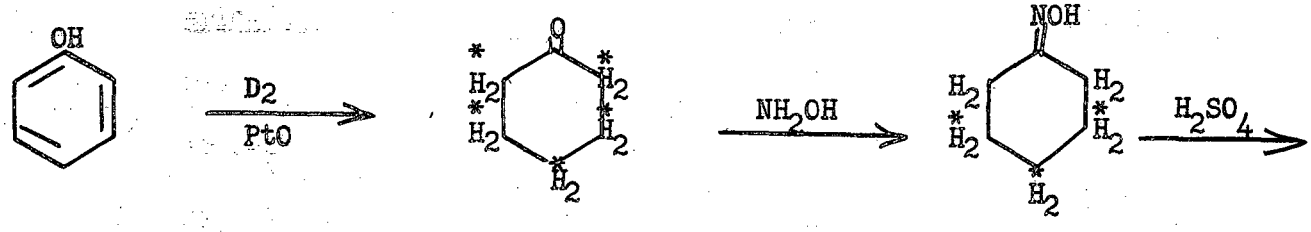
The modified Gabriel synthesis employing diethyl sodium phthalimidomalonate has been applied (15) to the preparation of DL-proline-N^{15}:

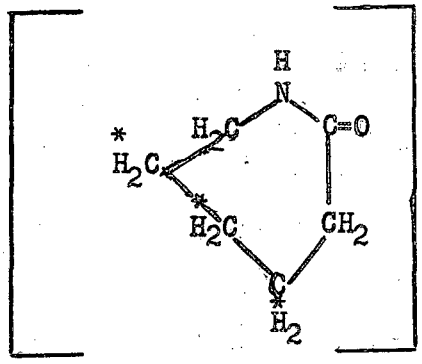


The Gabriel synthesis can be used to prepare an amino acid labeled with both nitrogen and hydrogen by employing an α-halo acid labeled with iso-

topic hydrogen. By this modification, deuterio-DL-leucine-N^{15} has been prepared (13) from deuterio-isocaproic acid with the use of the same sequence of reactions described in Section II for the singly labeled compound. The D and L forms of deuterio-leucine-N^{15} have been isolated (18,19) from the synthetic racemic mixture.

Deuterio-cyclohexanone, prepared by the catalytic reduction of phenol, has been used (20) to prepare DL-lysine-β,γ,δ-d-N^{15}:





1. Hydrolysis
2. Benzoylation

$\longrightarrow C_6H_5CONHCH_2(\overset{*}{C}H_2)_3CH_2CO_2H$

$\xrightarrow{P,\ Br_2} C_6H_5CONHCH_2(\overset{*}{C}H_2)_3CHBrCO_2H \longrightarrow C_6H_5CONHCH_2(\overset{*}{C}H_2)_3CHBrCO_2C_2H_5$

1. Phthalamide-N^{15} + CuO
2. Hydrolysis

$\longrightarrow H_2NCH_2(\overset{*}{C}H_2)_3CH(NH_2)CO_2H$

Labeled L-lysine was isolated from the racemic mixture.

The Knoop Reaction: - The use of the Knoop reaction for the preparation of amino acids labeled with isotopic hydrogen has been discussed in Section II. The reaction can be used equally well to introduce isotopic nitrogen by substituting ammonia-N^{15} for ordinary ammonia in the procedure. In this way, the following amino acids have been prepared from the corresponding keto acids: DL-alanine-N^{15} (13), DL-phenylalanine-N^{15} (13), DL-tyrosine-N^{15} (13), DL-norleucine-N^{15} (13), DL-aspartic acid-N^{15} (13,15), DL-glutamic acid-N^{15} (13) and DL-γ-phenyl-α-amino-N^{15}-butyric acid (2). The yields range from 40-85%, based on total ammonia taken; most of the ammonia remaining unconverted is recoverable as such from the liquors. From racemic mixtures, L-glutamic acid-N^{15} (15) and the D and L forms of γ-phenyl-α-amino-N^{15}-butyric acid (21) have been prepared.

An amino acid labeled with both hydrogen and nitrogen can be prepared by the Knoop reaction if isotopic hydrogen and $N^{15}H_3$ are used together in the procedure. By this device, α-ketoglutaric acid has been converted (22) to DL-glutamic acid-α,β-d-N^{15}.

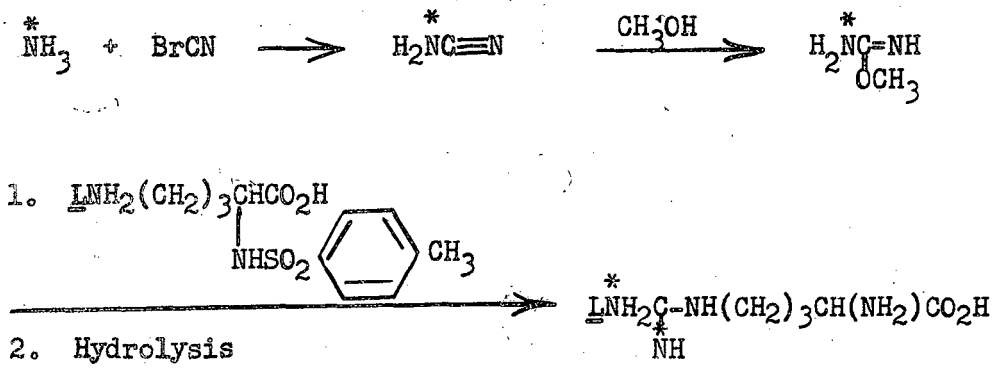
By a modification of the Strecker procedure, acetaldehyde has been converted to DL-alanine-N^{15} by treatment with sodium cyanide in the presence of isotopic ammonium chloride (15):

$$CH_3CHO \xrightarrow[\overset{*}{N}H_4Cl]{NaCN} CH_3CH(\overset{*}{N}H_2)COOH$$

L-alanine-N^{15} was isolated from the synthetic racemate.

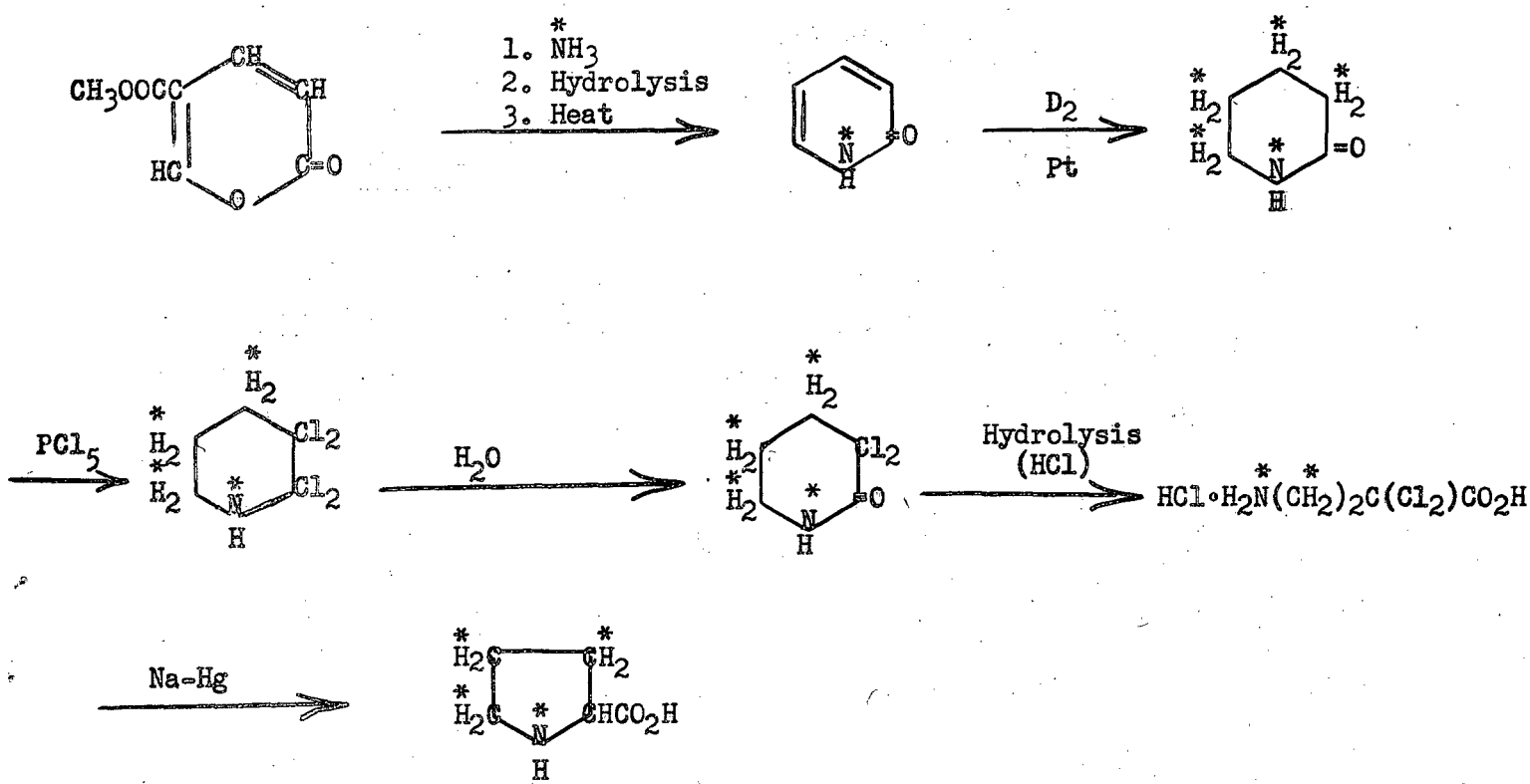
In addition to these general types, a few specialized syntheses have been carried through with isotopic materials.

L-arginine-N^{15} labeled in the amidine group has been prepared (16) by the following sequence:



Two nitrogen atoms in the guanidine group become labeled since they are rendered indistinguishable by tautomerism.

DL-proline-3,4,5-d-N^{15} has been prepared (12) by the following sequence:



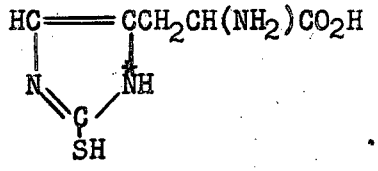
L-proline-3,4,5-d-N^{15} was isolated from the racemic mixture.

L-histidine-1-N^{15} has been prepared (23) by reaction of sodium thiocyanate-N^{15} with γ-ketoornithine, followed by treatment with ferric sulfate of the thiolhistidine so formed:

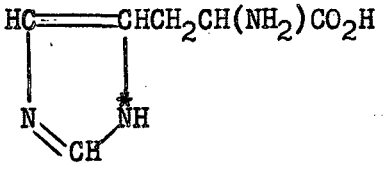
$$3\ \overset{*}{N}H_4NO_3 + 3\ CS_2 + 2\ Fe(OH)_3 + 6\ NaOH \xrightarrow[90\%]{}$$

$$3\ NaS\overset{*}{C}N + 2\ FeS + S + 3\ NaNO_3 + 2\ H_2O$$

$$LH_2NCH_2COCH_2CH(NH_2)CO_2H + NaS\overset{*}{C}N \longrightarrow$$



$$\xrightarrow{Fe_2(SO_4)_3}$$



The yield was 17% based on thiocyanate.

IV.

INTRODUCTION OF C^{13} AND C^{14} INTO AMINO ACIDS

The preparation of amino acids labeled with isotopes of carbon (C^{13} or C^{14}) requires the use of a greater variety of synthetic methods than does the synthesis of these compounds labeled with other isotopes; the usual isotopic starting material is either cyanide or carbonate.

The isotope C^{11}, with a half-life of 21 minutes, has not been used to label an amino acid, although it has been used for tracer studies on other types of compounds of biological interest. Because of the nature of the operations involved, it would be difficult to synthesize a labeled amino acid, carry out an experiment and make radioactivity measurements quickly enough to avoid losing most of the original activity by decay.

Cyanide, in which form isotopic carbon can be purchased or can be prepared from isotopic barium carbonate (24,25), is a particularly convenient material with which to begin the synthesis of an amino acid. It can be used directly in the Strecker reaction, or it can be treated with an aliphatic halogen compound to form a nitrile. The nitrile can then be hydrolyzed and the resulting carboxylic acid converted to an amino acid by one of the standard methods.

Thus, the methyleneaminoacetonitrile synthesis, a derivative of the Strecker procedure, has been used (25) to convert formaldehyde to glycine-1-C^{14} in 50% yield, based on cyanide.

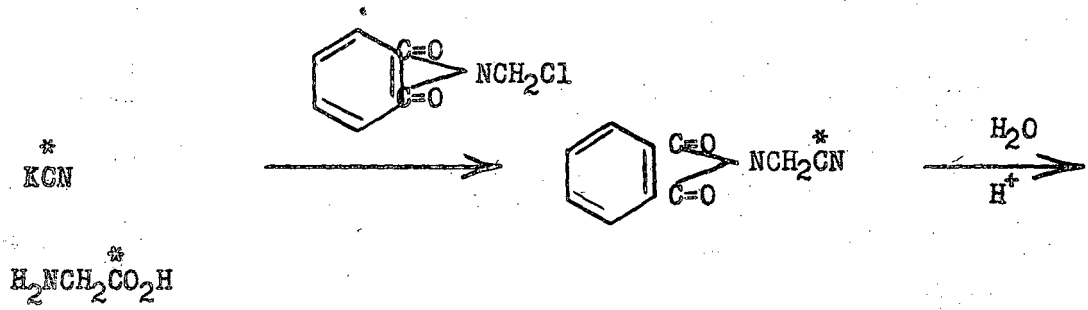
$$\overset{*}{K}CN \xrightarrow[NH_4Cl]{HCHO} HC{=}NCH_2\overset{*}{C}N \xrightarrow[H_2SO_4]{C_2H_5OH} (H_2NCH_2\overset{*}{C}N)_2H_2SO_4 \xrightarrow{Ba(OH)_2}$$

$$(H_2NCH_2\overset{*}{C}O_2)_2Ba \xrightarrow{H_2SO_4} H_2NCH_2\overset{*}{C}O_2H$$

Acetaldehyde has been converted (25,26) by the Strecker synthesis to DL-alanine-1-C^{14}. The yield, based on barium carbonate from which the cyanide was prepared, is 35%.

$$\overset{*}{H}CN \xrightarrow[CH_3CHO]{NH_3} CH_3CH(NH_2)\overset{*}{C}N \xrightarrow[H^+]{H_2O} CH_3CH(NH_2)\overset{*}{C}O_2H$$

Glycine-1-C^{13} has been prepared (27) in 81% yield, based on cyanide, by the reaction between chloromethylphthalimide and labeled cyanide, followed by hydrolysis of the phthalimidoacetonitrile so formed:



The same procedure has been used (26) to prepare glycine-1-C^{13}-N^{15} from chloromethyl phthalimide labeled with N^{15}.

A synthesis of DL-methionine-S^{35}-β,γ-C^{13} in which cyanide is used as the primary source of C^{13} is described in Section V.

Practically all of the procedures for introducing isotopic carbon which do not employ labeled cyanide as the starting material begin with isotopic carbon dioxide (barium carbonate).

Carbon dioxide has been converted to methyl iodide by three semi-micro techniques. The first (28) requires the use of high pressure equipment, but gives a yield of 84% against 76% for the second (29). The yield of methanol

based on carbon dioxide is 81% by the third method (30), which requires the least specialized equipment.

A. $\overset{*}{CO_2} \xrightarrow[\text{K-Cu-Al oxide}]{H_2} \overset{*}{CH_3OH} \xrightarrow{P,\ I_2} \overset{*}{CH_3I}$

B. $\overset{*}{CO_2} \xrightarrow{NaOH} NaH\overset{*}{CO_3} \xrightarrow[Pd]{H_2} H\overset{*}{CO_2}Na \xrightarrow{(CH_3)_2SO_4} H\overset{*}{CO_2}CH_3$

$\xrightarrow[\text{Cu chromite}]{H_2} \overset{*}{CH_3OH} \xrightarrow{HI} \overset{*}{CH_3I}$

C. $\overset{*}{CO_2} \xrightarrow{LiAlH_4} LiAl(OCH_3)_4 \xrightarrow{ROH} \overset{*}{CH_3OH} \xrightarrow{P_4,\ I_2} \overset{*}{CH_3I}$

Methyl iodide-C^{14} so prepared can be used (29) to convert S-benzyl-homocystine to L-methionine-methyl-C^{14} in 84% yield, based on methyl iodide:

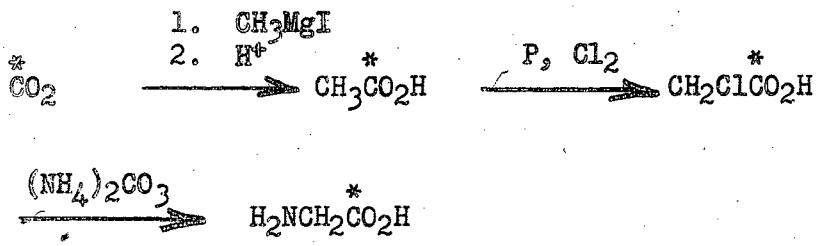
$$\overset{*}{CH_3I} \xrightarrow[\text{Liq. } NH_3]{Na\ +\ C_6H_5CH_2SCH_2CH_2CH(NH_2)CO_2H} \overset{*}{CH_3}SCH_2CH_2CH(NH_2)CO_2H$$

Methyl iodide-C^{14} can also be used (31,32) to prepare glycine-2-C^{14}. The iodide is converted to the Grignard reagent, which is carbonated with ordinary carbon dioxide to form acetic acid-2-C^{14}. This is converted to chloroacetic acid and aminated. The yield is about 55% based on isotopic barium carbonate used as starting material.

The synthesis of glycine-2-C^{14} just described illustrates the use of the carbonation of a Grignard reagent to form a carboxylic acid. By the substitution of isotopic for ordinary carbon dioxide, this reaction can be adapted to the

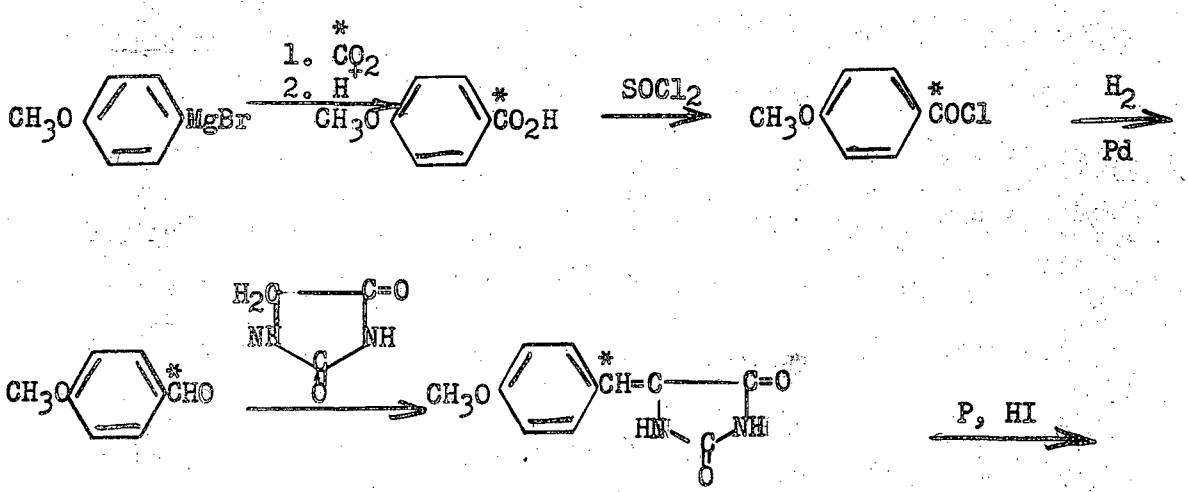
synthesis of carboxyl-labeled acids and is widely used for the purpose. Various procedures for converting carboxylic acids to amino acids can then be applied.

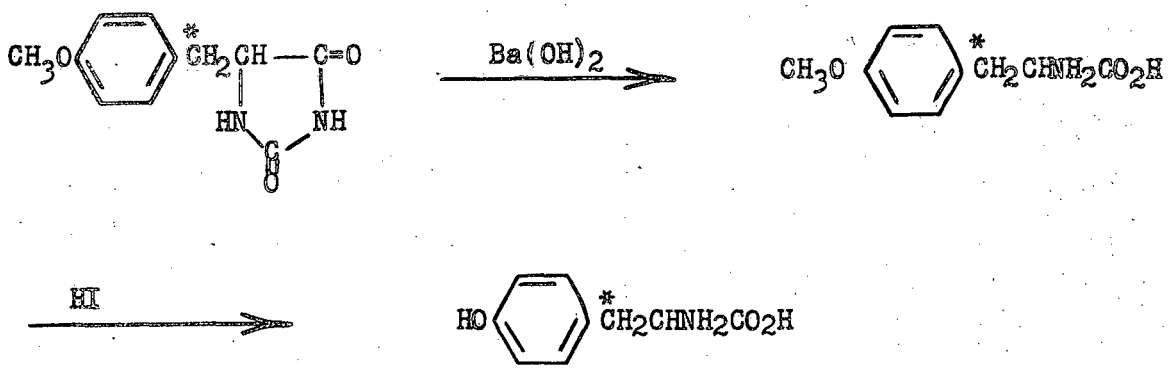
Thus, carbon dioxide-C^{14} has been converted to glycine-1-C^{14} in 60% yield from barium carbonate (32,33).



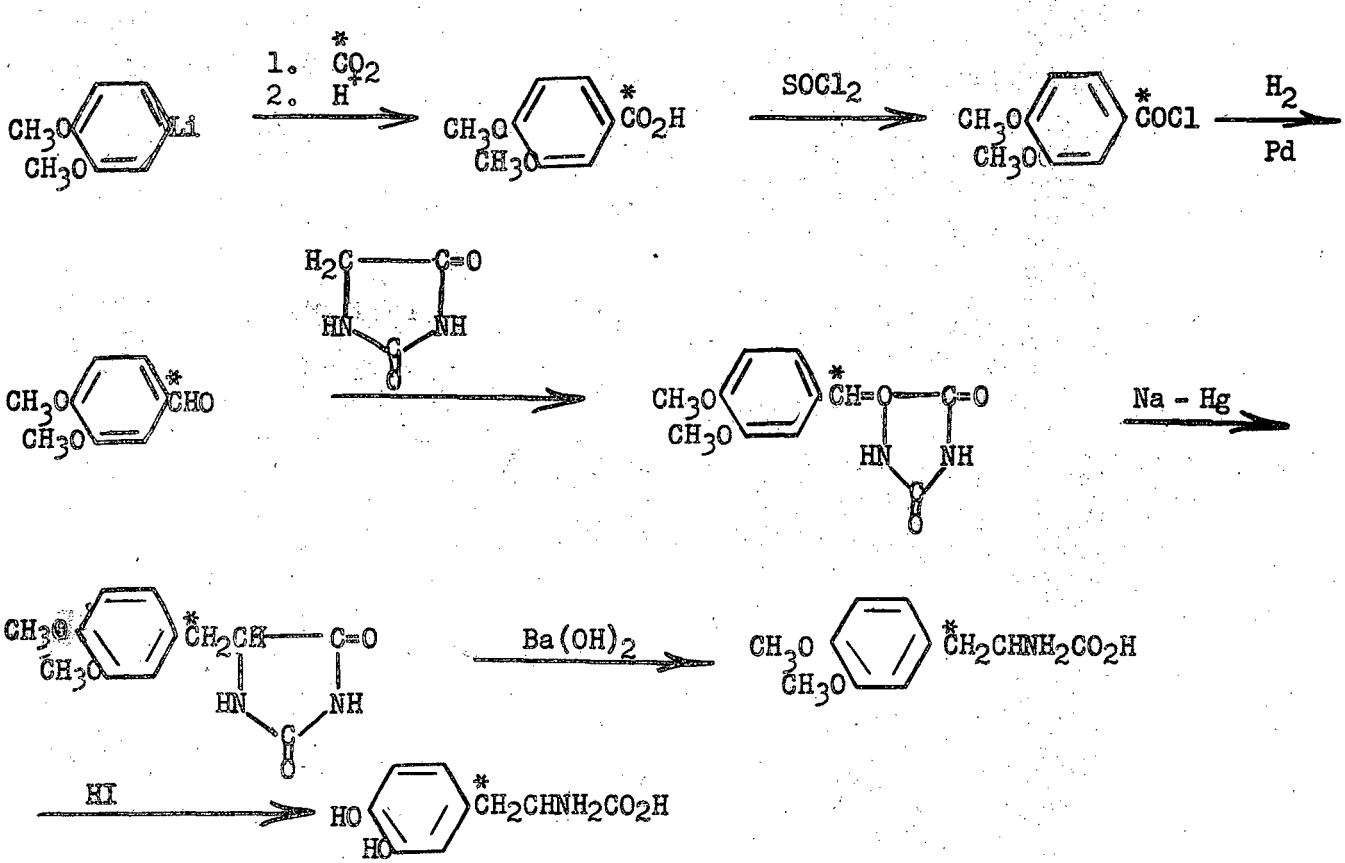
A somewhat better yield (69% based on barium carbonate) has been obtained (27) in the synthesis of glycine-1-C^{13} by conversion of the labeled acetic acid to ethyl bromoacetate and application of the Gabriel synthesis.

A carboxyl-labeled intermediate can sometimes be used to prepare an amino acid labeled in a position other than the carboxyl group. Thus, DL-tyrosine-β-C^{14} can be prepared (34) in 19% yield (based on barium carbonate) from carboxyl-labeled p-anisic acid by conversion to p-anisaldehyde followed by condensation with hydantoin and reduction and hydrolysis of the p-methoxybenzalhydantoin so formed:

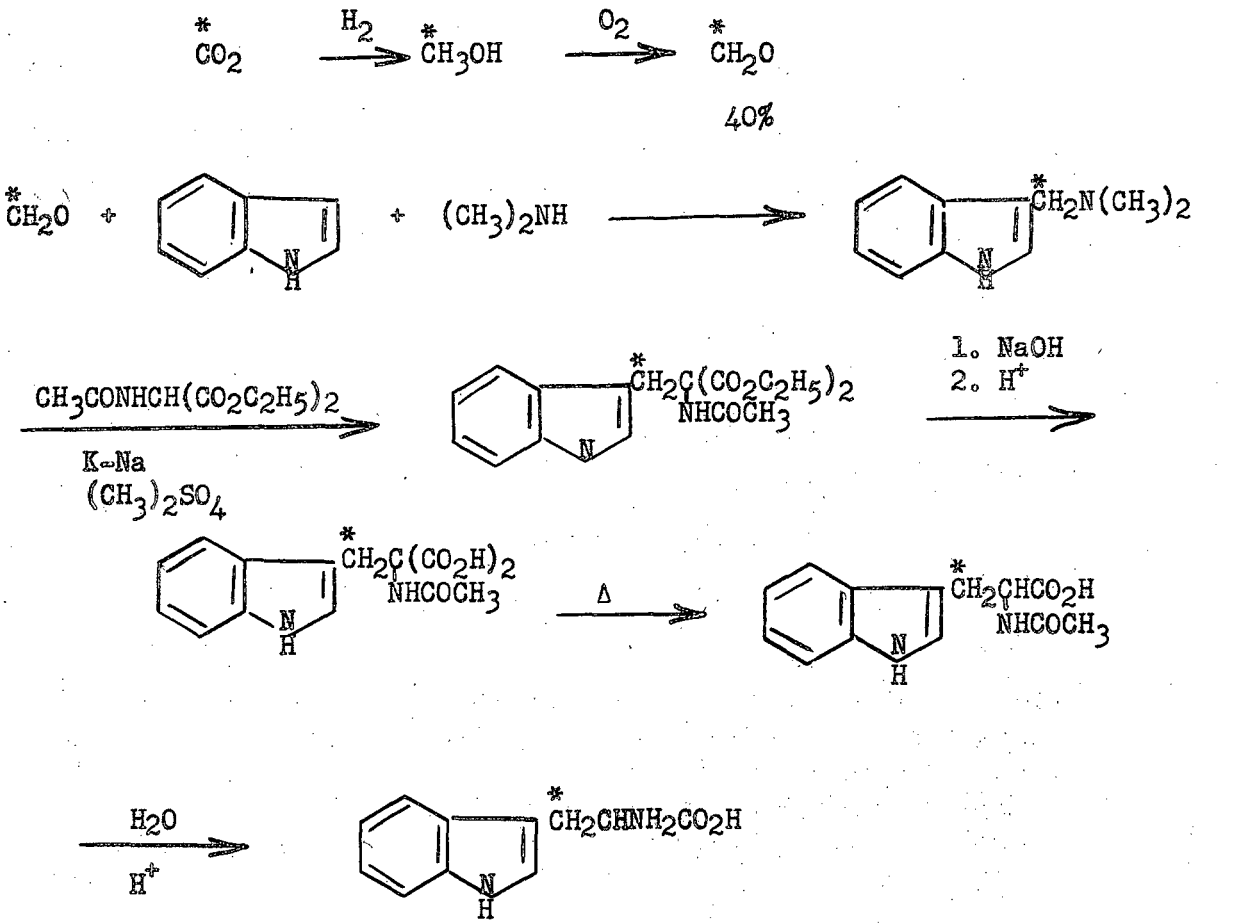




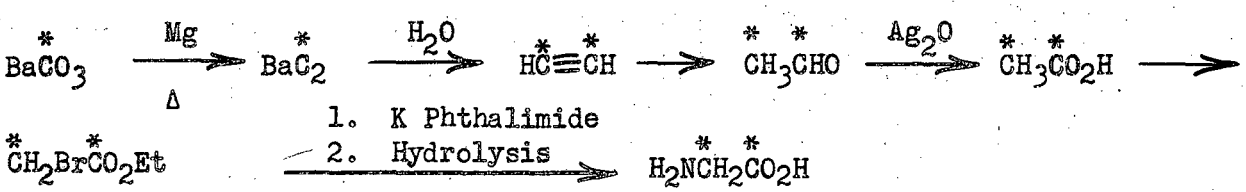
DL-3,4-Dihydroxyphenylalanine-β-C^{14} has been prepared (35) from barium carbonate in 18% yield by a scheme similar to that used for tyrosine, except that a lithium alkyl was used instead of a Grignard reagent in the initial carbonation (35):



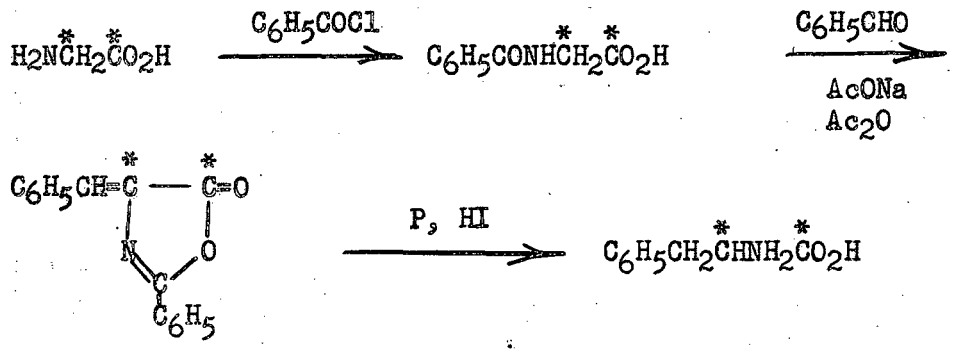
Formaldehyde prepared by the oxidation of methanol (36) can be used as the starting point for the synthesis of DL-tryptophan-β-C^{14}. The yield is 22% based on carbon dioxide.



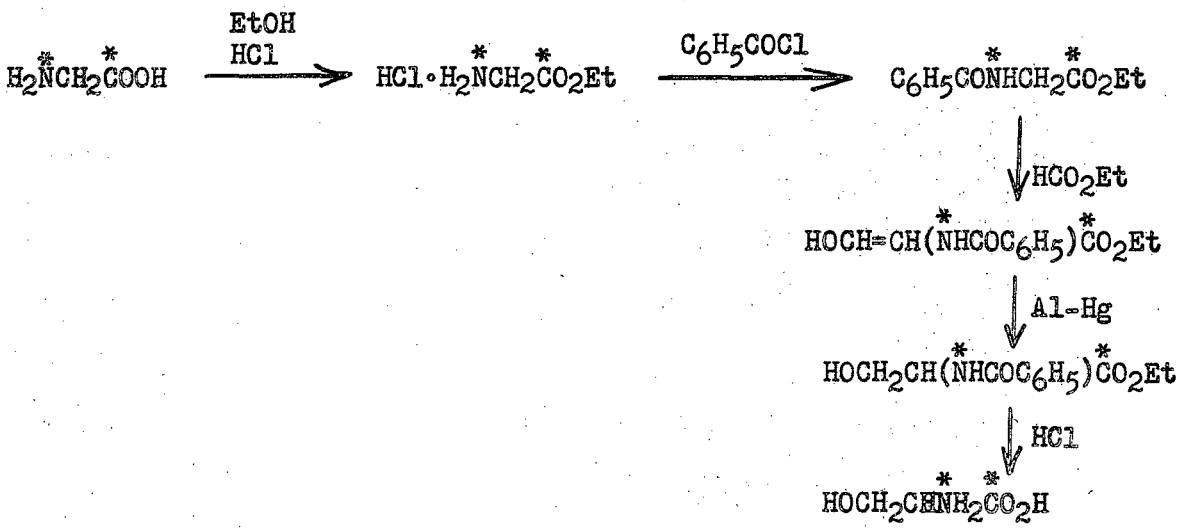
Acetylene prepared from isotopic barium carbide is a useful intermediate for the preparation of compounds labeled in two positions. Glycine-1,2-C^{14} has been synthesized from this intermediate by the following sequence (4):



Glycine itself has been used as an intermediate in the synthesis of other amino acids. DL-phenylalanine-α,carboxyl-C^{14} has been prepared (4) from doubly labeled glycine-C^{14} by the azlactone synthesis. The overall yield based on glycine is 50%.



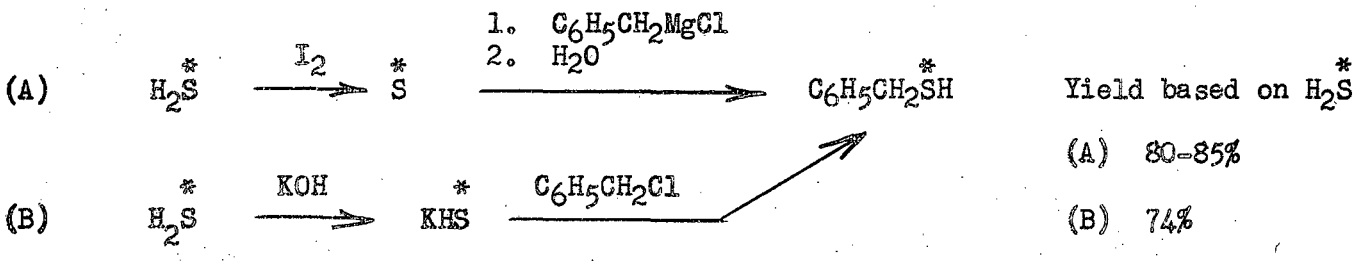
Labeled glycine has also been used as an intermediate in the synthesis (15) of DL-serine-1-C^{13}-N^{15}:



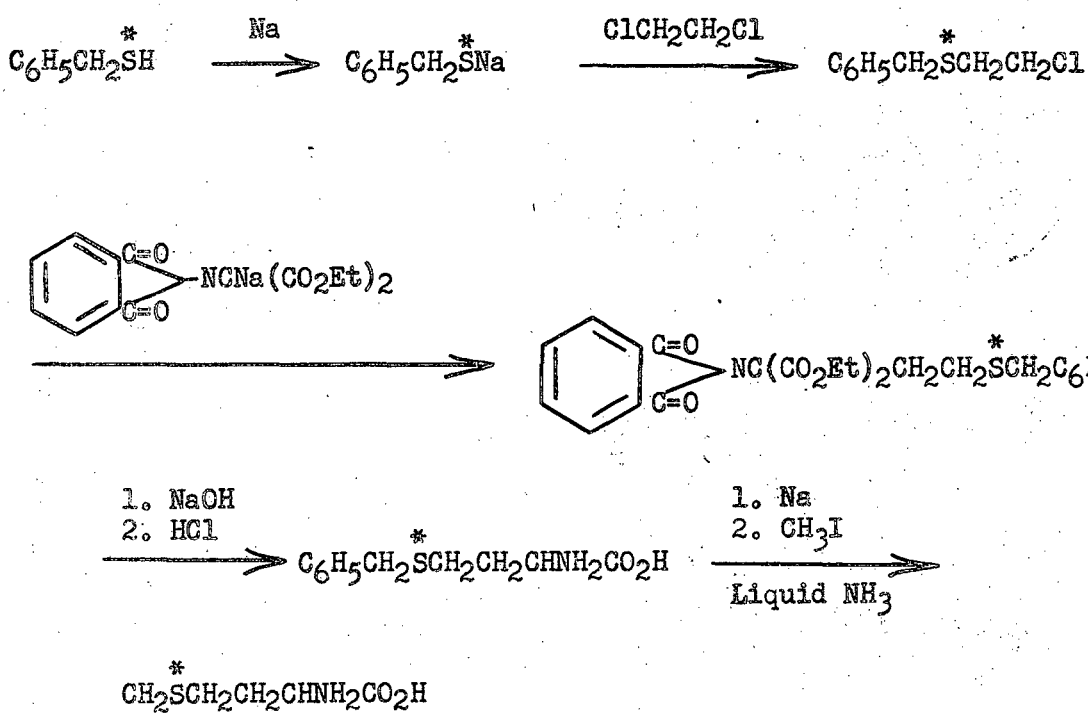
V.

INTRODUCTION OF S^{34} AND S^{35} INTO AMINO ACIDS

In all of the methods so far applied to prepare amino acids with marked sulfur atoms, labeled benzyl mercaptan has served as an intermediate. Labeled benzyl mercaptan can be prepared in two ways (37,38,39, 40):



DL-methionine-S^{35} has been prepared (38,40) by continuing as follows:



The overall yield is about 20% based on isotopic sulfur used to prepare the hydrogen sulfide.

DL-homocystine-S^{35} has been prepared (40) by the scheme just described for methionine, except that methyl iodide is not added in the last step; instead oxygen is bubbled through the reduced solution. The yield is about 25% based on sulfur.

In an improved synthesis of methionine and cystine the benzyl mercaptan is condensed with ethyl-Δ-chloro-α-benzamidobutyrate (39) or ethyl-β-chloro-α-benzamidopropionate (41), respectively; hydrolysis of the condensation product gives benzylhomocysteine (A) or benzylcysteine (B):

(A) $C_6H_5CH_2\overset{*}{S}Na \xrightarrow{ClCH_2CH(NHCOC_6H_5)CO_2Me} C_6H_5CH_2\overset{*}{S}CH_2CH_2CH(NHCOC_6H_5)CO_2Et$

$$\xrightarrow[H^+]{H_2O} C_6H_5CH_2\overset{*}{S}CH_2CH_2CHNH_2COOH$$

(B) $C_6H_5CH_2\overset{*}{S}K \xrightarrow{ClCH_2CH(NHCOC_6H_5)CO_2Me} C_6H_5CH_2\overset{*}{S}CH_2CHNH(COC_6H_5)CO_2Me$

$$\xrightarrow[H^+]{H_2O} C_6H_5CH_2\overset{*}{S}CH_2CHNH_2CO_2H$$

The benzyl group is then removed as before with sodium in liquid ammonia, followed by methylation or oxidation, respectively.

DL-cystine-S^{35} has also been prepared (40) by the following method:

$$C_6H_5CH_2\overset{*}{S}H \xrightarrow[HCl]{CH_2O} C_6H_5CH_2\overset{*}{S}CH_2Cl \xrightarrow[2.\ NaOH\quad 3.\ HCl]{1.\ \text{phthalimide-}NCNa(CO_2Et)_2} C_6H_5CH_2\overset{*}{S}CH_2CHNH_2CO_2H$$

$$\xrightarrow[\text{Liquid } NH_3]{1.\ Na\quad 2.\ O_2} HO_2CCHNH_2CH_2\overset{**}{S}SCH_2CHNH_2CO_2H$$

The yield is 17% based on benzyl mercaptan.

The doubly labeled DL-methionine-S^{34}-β,γ-C^{13} has been prepared (37) by same method, employing ethylene chloride-C^{13} for the condensation with labeled benzyl mercaptan. The labeled ethylene chloride was prepared in 56% yield, based on cyanide, in the following way:

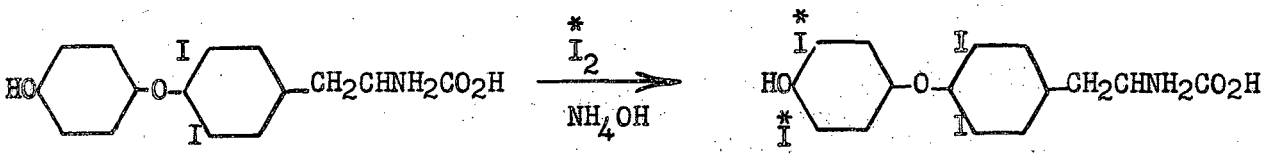
$$\overset{*}{NaCN} \xrightarrow{CH_3I} CH_3\overset{*}{C}N \xrightarrow{H_2} CH_3\overset{*}{C}H_2NH_2 \xrightarrow{CH_3I} CH_3\overset{*}{C}H_2N(CH_3)_3I$$

$$\xrightarrow{Ag_2O} CH_3\overset{*}{C}H_2N(CH_3)_3OH \xrightarrow{\Delta} \overset{*}{C}H_2{=}\overset{*}{C}H_2 \xrightarrow{Cl_2} CH_2Cl\overset{*}{C}H_2Cl$$

VI.

INTRODUCTION OF IODINE^{131} INTO AMINO ACIDS

In addition to preparation by extraction from the thyroid glands of animals given radioactive inorganic iodine, thyroxine has been prepared synthetically with two labeled iodine atoms (42) as follows:



The radioactive iodine was obtained as sodium iodide. By treatment of this compound with iodate, free iodine was prepared and this was used in the synthesis.

Labeled thyroxine has also been prepared (43) by direct exchange between thyroxine and radioactive iodide at pH 5.

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