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Synthesis of Several C^{14}-Labeled DL-Alanines

R. Ostwald, P. T. Adams and B. M. Tolbert

October 13, 1950

Berkeley, California

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SYNTHESIS OF SEVERAL C^{14}-LABELED DL-ALANINES

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October 13, 1950

ABSTRACT

The synthesis of the three mono-C^{14}-labeled DL-alanines and necessary intermediates to prepare them via propionic acid is described. Small scale procedures were used throughout. Ion exchange columns and vacuum sublimation were used to give high purity amino acids in good yield on a small scale.

(*) Supported by a grant to Prof. D. M. Greenberg, University of California, from the American Cancer Society (recommended by the Committee on Growth of the National Research Council).

(**) The work described in this paper, was in part, supported by the Atomic Energy Commission.

SYNTHESIS OF SEVERAL C^{14}-LABELED DL-ALANINES

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The synthesis of DL-alanine singly labeled with C^{14} in each of the three positions was undertaken in conjunction with some of the biological studies of this group and of the division of Biochemistry of the University of California. In the course of this work, procedures for the small-scale synthesis of several important intermediates were developed, including the 2- and 3-labeled propionic acids. Although both 1- and 2-labeled alanine have been prepared before[1,2,3,4] other methods with much lower yields have been used.

The reaction schemes used were as follows:

(1) $$C^{14}O_2 \xrightarrow[H^+]{C_2H_5MgBr} CH_3CH_2C^{14}O_2H \xrightarrow{NaOH} CH_3CH_2C^{14}O_2Na$$

(2) $$CH_3C^{14}O_2Na \xrightarrow{HCl\ (g)} CH_3C^{14}O_2H \xrightarrow{LiAlH_4} CH_3C^{14}H_2OH$$

$$\xrightarrow{PBr_3} CH_3C^{14}H_2Br \xrightarrow[CO_2,\ H^+]{Mg} CH_3C^{14}H_2CO_2H \xrightarrow{NaOH} CH_3C^{14}H_2CO_2Na$$

(*) Supported by a grant to Prof. D. M. Greenberg, University of California, from the American Cancer Society (recommended by the Committee on Growth of the National Research Council.)

(**) Supported, in part, by the Atomic Energy Commission.

1. S. Gurin and D. W. Wilson, Feder. Proc. 1, 114 (1942)

2. R. B. Loftfield, Nucleonics, 1, No. 3, 54 (1947)

3. D. Frank, R.B. Loftfield and W.W. Miller, Science, 106, 544 (1947)

4. J. Baddiley and J. Ehrenvöörd and H. Nilsson, J. Bio. Chem., 178, 399 (1949)

(3) $$C^{14}H_3CO_2Na \xrightarrow[LiAlH_4]{HCl(g)} C^{14}H_3CH_2OH \xrightarrow{PBr_3} C^{14}H_3CH_2Br$$

$$\xrightarrow[\substack{CO_2 \\ H^+}]{Mg} C^{14}H_3CH_2CO_2H \xrightarrow{NaOH} C^{14}H_3CH_2CO_2Na$$

(4) $$CH_3CH_2CO_2Na \xrightarrow{HCl(g)} CH_3CH_2CO_2H \xrightarrow[CH_3CH_2COCl]{Br_2, P_4, I_2} CH_3CHBrCOX$$

$$\xrightarrow{H_2O} CH_3CHBrCO_2H \xrightarrow[(NH_4)_2CO_3]{NH_4OH} CH_3CHNH_2CO_2H$$

It was observed that in the reduction of the acetic acid with lithium aluminum hydride in diethyl carbitol solvent there occurred a marked and erratic dilution of the labeled ethanol with inactive material.

To insure that the solvent itself was originally free from ethanol, it was treated with lithium aluminum hydride, filtered and fractionally distilled. The fraction boiling at 72°/10.5 mm. was used for the syntheses and for all experimental work. Attempts to identify ethanol in the solvent after treatment with lithium aluminum hydride or in the $LiAlH_4$ itself were unsuccessful. The presence of carbon-containing compounds in the lithium aluminum hydride was demonstrated, but its analysis (C; 0.95, 0.59%) in the two samples used) showed this source to be insufficient to account for the inactive halide formed in the subsequent reaction.

After treating the diethyl carbitol with phosphorus tribromide under the conditions of the bromination, 0.18 g. of product was isolated. When an identical amount of solvent was first treated with lithium aluminum hydride, then brominated, the product was 0.33 g. Even when the greatest of care was

taken to exclude diethyl carbitol from the bromination of synthetic ethanol, yields in excess of theoretical were still found.

Methanol was prepared by lithium aluminum hydride reductions of $C^{14}O_2$ in a check reaction and converted to the iodide. Sodium acetate was prepared from the methyl iodide[5]. The equivalent weight of the acetates so prepared was as much as 8% high, whereas that of sodium propionate made from ethyl bromide prepared in the manner described above varied a maximum of 0.6% from theoretical in six runs.

On the basis of this work, it was concluded that probably some lithium compound caused splitting of the diethyl carbitol under the conditions of the preparation to give ethanol and thus cause the effects described.

The purification of the alanine was achieved by a novel combination of steps which gave a high purity material on a small scale with a minimum loss of material due to manipulation. In this procedure the alanine was first absorbed on a cation exchange resin and the anions (chloride, phosphate and bromide ions) washed out. The alanine was then eluted with ammonia to give an alanine-ammonia solution. When this eluate was evaporated to dryness, fairly pure, salt-free alanine was left. By high vacuum sublimation of this material a very pure product was obtained.

The identity and purity of the propionic acids was checked by equivalent weight determinations. The chemical identity and radiopurity of the derived amino acids was checked by paper chromatography and radioautography of the paper chromatograms. After this, the papers were sprayed with ninhydrin.

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5. B. M. Tolbert, J. Biol. Chem., 173, 205 (1948)

These experiments showed that only one amino acid, alanine, was present in each compound and that the ninhydrin spot corresponded with the radioautograph spot.

The importance of this check can be shown by the following unsuccessful experiment. The preparation of alanine-3-C^{14} was first attempted by condensation of diethyl acetamidomalonate with labeled methyl iodide. It was found that an appreciable amount of a radioactive contaminant was present in the alanine thus formed, which had amino acid like properties and could not be effectively separated on a small scale. This impurity, believed to be sarcosine (N-methyl glycine), would not be easily detected in alanine by ordinary analytical methods (elemental analyses, recrystallization, etc.) and indicates the importance in the small-scale radiopreparations of special purity tests such as the paper chromatograms and radioautographs just mentioned.

It is interesting to note that small quantities of alanine may be decarboxylated by bacterial action if left standing in unsterilized solution at room temperature. Thus, 59 mg. of alanine-1-C^{14} was aerated for three days in aqueous solution and the carbon dioxide was trapped in sodium hydroxide and precipitated as barium carbonate. It was found that of the 2.43×10^6 dis./min. put into some 75 ml. water only 1.69×10^6 dis./min. were found in the solution after aeration and 0.69×10^6 dis./min. were found in the barium carbonate precipitated from the sodium hydroxide bubblers.

Experimental

Sodium propionate-1-C^{14}. -- Thirty millimoles of methyl magnesium iodide was carbonated with 20 mmoles of $C^{14}O_2$ (from 3.94 g. $BaC^{14}O_3$, 5.02 μc/mg.[6]).

6. "Isotopic Carbon", Calvin, et.al., John Wiley and Sons, Inc., New York, New York (1949), pp. 178-179.

The yield was 1.87 g. of sodium propionate-1-C^{14} (specific activity 10.4 μc/mg.) which is a 97.8% yield.

α-Bromopropionic-1-C^{14} acid. -- Dry sodium propionate (937 mg. 9.87 mc. from the above preparation was placed in a gas-solid reactor[7]. This bulb was then evacuated to about 10 microns pressure and filled with an excess (one-half atmosphere) of dry purified HCl gas. The solid sodium propionate was then heated gently over a Bunsen burner. After the exchange reaction was complete the mixture of propionic acid and excess HCl was distilled *in vacuo* into a large trap cooled with liquid nitrogen. The cooling bath was then changed to a Dry Ice-isopropyl alcohol mixture, and the excess HCl pumped off. In preliminary runs it was established that the salt residue contained less than 0.1% of the initial activity and no demonstrable amounts of propionic acid. It was also shown that the propionic acid so prepared contained 3-5% of water.

The propionic-1-C^{14} acid was distilled into the bromination vessel[8] which contained 0.04 g. red phosphorus, 0.02 g. iodine and 0.2 cc. propionylchloride. The mixture was allowed to reflux on the steam bath for one-half hour to destroy the water, 1.5 cc. bromine was added dropwise and refluxing was continued for three hours[9]. The low temperature condenser was kept at isopropyl-alcohol-Dry Ice temperature.

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7. "Isotopic Carbon", Calvin, *et.al.*, John Wiley and Sons, Inc., New York, New York (1949), pp. 162.

8. B. M. Tolbert, F.C. Christenson, F.N.H. Chang and P.P.T. Sah, J. Org. Chem., 14, Fig. 3, p. 528 (1949)

9. J.C. Eck and C.S. Marnel, "Organic Synthesis", Collective Vol. II, p. 74 (1943)

After sweeping out the excess bromine with a slow stream of air, the mixture of α-bromopropionic-1-C^{14} acid and α-bromopropionyl-1-C^{14} bromide was cooled and hydrolyzed by slow addition of 2 cc. of water.

Alanine-1-C^{14}. -- The amination was performed in a three-necked flask by slow addition of α-bromopropionic-1-C^{14} acid to a mixture of 6 g. ammonium carbonate and 15 cc. concentrated ammonia hydroxide[10,11]. The reaction mixture was kept at 60°C for six hours and then vacuum distilled to dryness. The distillate contained 1-2% of the starting activity.

The crude alanine-1-C^{14} was purified by (a) passage through an ion exchange resin column and (b) high vacuum sublimation.

A glass column (30 cm. x 2 cm.) filled with 60 cc. of Dowex 50 resin (20-40 mesh) was treated by cycling it to exhaustion three or four times with 2 N sodium hydroxide solution and 2 N hydrochloric acid, respectively, ending with the acid. All the excess acid was washed out thoroughly with water, and then the 600-900 mg. of crude alanine-1-C^{14}, dissolved in a minimum amount of water, was added to the column, followed by 500 cc. of water to elute the anions. The alanine was eluted with 250 cc. of 1.5 N ammonium hydroxide, followed by 250 cc. of water. The resin was regenerated with 2 N hydrochloric acid. The water effluate, ammonium hydroxide eluate and hydrochloric acid regeneration solution were each evaporated to dryness and were found to contain 3%, 88% and

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10. N.D. Cheronis and K. H. Spitzmueller, J. Org. Chem., 6, 349 (1941)

11. J.M. Orton and R.W. Hill, "Organic Synthesis", Collective Vol. I, 300 (1941)

5%, respectively, of the activity put on the resin.

In order to remove traces of ammonia the dry residue of the ammonium hydroxide eluate was made slightly alkaline with sodium hydroxide and evaporated to dryness. After redissolving and adjusting the pH to 6.8 the solution was transferred to the lower part of a sublimation apparatus with a one centimeter sublimation gap, evaporated to dryness and sublimed at one micron pressure and 160-200° for two hours with the cold finger at liquid nitrogen temperature. The sublimation residue was redissolved (pH 8.5), adjusted to pH 6.8, dried and resublimed. This final residue contained 4% of the initial crude alanine activity.

The yield of alanine-1-C^{14} was 802 mg. with a specific activity of 9.9 μc/mg. (theoretical, 9.11 μc/mg.). This represents a radiochemical yield of 80.5% based on sodium propionate-1-C^{14} used.

Ethyl-1-C^{14} Bromide. -- Acetic-1-C^{14} acid was prepared from 1.602 g. (29.2 mc.) of sodium acetate-1-C^{14} (6) using dry HCl gas as in the previous procedure for preparing propionic acid. The product was distilled *in vacuo* into a dropping funnel which contained 5 ml. of repurified diethyl carbitol. The funnel was attached to a three-necked conical flask which contained 1.16 g. lithium aluminum hydride in 35 ml. diethyl carbitol and to which was also attached a U-trap immersed in a Dry Ice bath (Fig. 1)[12,13]. The acid solution was dropped slowly onto the lithium aluminum hydride solution with occasional

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12. R.F. Nystrom and W. G. Brown, J. Am. Chem. Soc., 69, 1197; 2548 (1947)

13. R. F. Nystrom, W. H. Yanko and B. G. Brown, J. Am. Chem. Soc., 70, 441 (1948)

shaking and cooling. After addition of the acid was completed, the dropping funnel was rinsed with several small portions of the solvent and the solution was allowed to stand with occasional shaking for 30 minutes. The lithium aluminum hydride solution was decomposed by the slow addition of 50 ml. of N-butyl carbitol and the system swept free of hydrogen by a slow nitrogen stream.

The ethanol-1-C^{14} thus produced was distilled into the cold trap using a nitrogen sweep. Alternating periods of boiling were used to insure complete exchange between the excess N-butyl carbitol and the lithium ethanolate. Prolonged boiling without gas sweep may produce enough hydrogen to cause an explosion. The non-volatile distillation residue contained 2.9% of the initial activity.

The cold trap containing the ethanol and about 5 ml. of the solvent was attached to the vacuum line and subjected to two vacuum distillations to reduce the amount of carbitol present. The ethanol was then distilled into a small flask and converted to ethyl-1-C^{14} bromide with phosphorus tribromide[4].

The chemical yield of purified ethyl bromide was 2.49 g.; this represents about 50% radio dilution because of splitting of the diethyl carbitol by lithium compounds with the formation of ethanol.

Sodium propionate-2-C^{14}. -- The ethyl-1-C^{14} bromide thus prepared was converted to the Grignard reagent and this compound carbonated with inactive CO_2[1]. The yield of dry sodium propionate-2-C^{14} was 1.58 g. (19.8 mc.) of specific activity 12.5 μc/mg. or a radiochemical yield of 68%.

Alanine-2-C^{14}. -- Fifteen millimoles of this sodium propionate-2-C^{14} was converted to alanine as previously described. The radiochemical yield was 75%

based on the propionate used, and the specific activity of the product was 4.3 μc/mg.

Sodium propionate-3-C^{14}. -- As previously described 1.229 g. (13.2 mc.) sodium acetate-2-C^{14} was reduced with lithium aluminum hydride in diethyl carbitol solution and the alcohol produced converted to the bromide to give 1.92 g. of ethyl-2-C^{14} bromide. This labeled ethyl bromide was then converted to the Grignard reagent and carbonated. In this manner, 1.225 g. (6.51 mc.) of sodium propionate-3-C^{14} was produced, which represented a radiochemical yield of 49.5% based on the acetate used to begin the synthesis.

Alanine-3-C^{14}. -- Twelve millimoles of this sodium propionate-3-C^{14} was converted to alanine, as previously described. The radiochemical yield was 72% based on the sodium propionate used and the specific activity of the product was 5.74 μc/mg.

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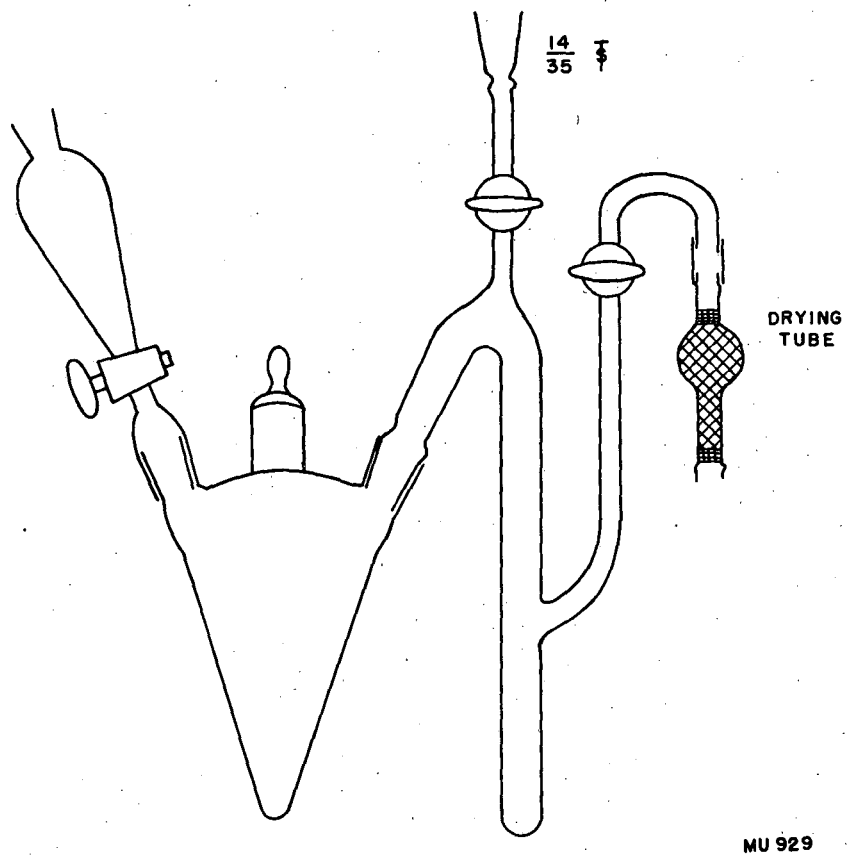
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