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THE SYNTHESIS OF LEUCINE AND SEVERAL BRANCHED CHAIN FATTY ACIDS LABELED WITH CARBON 14 IN VARIOUS POSITIONS

H. Hauptmann, P. T. Adams and B. M. Tolbert

October 2, 1950

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ABSTRACT

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October 2, 1950

(*) Rockefeller Fellow. While on leave from the Faculdade de Filosofía, Ciências e Letras, Universidade de São Paulo, São Paulo, Brasil.

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

ABSTRACT

The following compounds have been synthesized labeled with C^{14}. The yields, based on $C^{14}O_2$ used, are indicated in parentheses: Sodium isobutyrate-1-C^{14} (97.5); sodium isovalerate-1-C^{14} (96.0); sodium isocaproate-1-C^{14} (95.5); sodium isocaproate-2-C^{14} (39); DL-leucine-1-C^{14} (43.5); and DL-leucine-2-C^{14} (29.3). A novel method was developed for the preparation of alcohols from intermediate weight fatty acids and by this method isoamyl bromide-1-C^{14} was prepared in 73% yield based on sodium isovalerate-1-C^{14} used.

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The synthesis of a number of branched chain fatty acids and leucine labeled with C^{14} in various positions was undertaken in order to study their metabolism. The biological work will be described elsewhere. In this paper the preparation of these compounds is detailed.

By carbonation of the corresponding alkyl Grignard, three carboxyl-labeled acids were prepared: sodium isobutyrate-1-C^{14}, sodium isovalerate-1-C^{14} and sodium isocaproate-1-C^{14}:

$$RMgBr + C^{14}O_2 \xrightarrow[(2)\ H^+]{(1)\ H_2O} RC^{14}O_2Na$$
$$(3)\ NaOH$$

Sodium isocaproate-2-C^{14} was prepared from isovaleric acid-1-C^{14} via the alcohol, bromide and nitrile by a method that involved a novel approach to the problem of preparing pure alcohols and halides in good yields on a small scale.

$$(CH_3)_2CH_2CH_2C^{14}O_2Na \xrightarrow[280^\circ]{\text{cetyl bromide}} (CH_3)_2CH_2CH_2C^{14}O_2C_{13}H_{33}$$

$$(CH_3)_2CH_2CH_2C^{14}H_2OH \xrightarrow[\text{copper chromite}]{H_2} (CH_3)_2CH_2CH_2C^{14}H_2OH \xrightarrow{PBr_3} (CH_3)_2CH_2CH_2C^{14}H_2Br$$

$$(CH_3)_2CH_2CH_2C^{14}H_2Br \xrightarrow[(2)\ OH^-]{(1)\ KCN} (CH_3)_2CH_2CH_2C^{14}H_2CO_2Na$$

The cetyl ester was prepared in high yields (> 95%) by the direct reaction of sodium salt of the acid and the cetyl bromide. This product could then be reduced over copper chromite to give a mixture of the two alcohols. The large differences in the boiling point of these two compounds made possible the easy isolation of the low-boiling component in excellent yields in a relatively pure state.

The conversion of the isoamyl bromide to the isocaproic acid was first attempted by formation of the Grignard of the halide, followed by carbonation at - 25°C. The yields, however, were very low (about 40%) so the method via the nitrile which gave yields of 55-65% was used.

By bromination and amination of the corresponding labeled isocaproic acid, DL-leucine-1-C^{14} and DL-leucine-2-C^{14} were prepared:

$$(CH_3)_2CH_2(CH_2)_2CO_2H \xrightarrow{Br_2} (CH_3)_2CH_2CH_2CHBrCO_2H$$

$$(CH_3)_2CH_2CH_2CHBrCO_2H \xrightarrow{NH_3\ (liq.)} (CH_3)_2CH_2CH_2CHNH_2CO_2H$$

The radio purity of the amino acids was established by two-dimensional paper chromatography (1) using a butanol-propionic acid-water mixture in one

(1) A.A. Benson, et.al., J.Am.Chem.Soc. 72, 1710 (1950)

direction and phenol-water in the other. Radioautographs of the paper chromatogram showed only one radioactive spot and when the paper itself was sprayed with ninhydrin only one amino acid spot was observed. The identity of the acids was checked by equivalent weight determinations, and their radio purity established by one-dimensional paper chromatograms in water-ammonia-propanol solutions.

The authors wish to thank Prof. M. Calvin and Prof. R.T. Arnold for their help and encouragement in this work.

Experimental Procedure

The three carboxyl-labeled acids were prepared in the same manner using the same molal quantities of reagents. Thirty millimoles of the corresponding alkyl magnesium bromide in 20-50 ml. ether was added by means of a syringe to a conical reaction flask equipped with a stirrer (2) and connected to a vacuum line (3). The $C^{14}O_2$ was generated from 20 mmoles of $BaC^{14}O_3$ (3.94 g.) and dried by passing the gas through a Dry Ice-cooled spiral trap. This was then added *in vacuo* to the Grignard solution at -20°C with stirring. After one-half to one hour, the carbonation assembly was transferred to a hood, opened, and an excess of 6 N sulfuric acid and enough silver sulfate added to precipitate all the free bromide ions (a 20-30% excess was used). The flask was then fitted with a distillation head,

(2) B.M. Tolbert, W.G. Dauben and J.C. Reid, Anal.Chem., 21, 1014 (1949)

(3) M.Calvin, et.al., "Isotopic Carbon", John Wiley and Sons, Inc. New York (1949), p. 142, 179.

condenser and steam inlet and the product steam distilled. After titration to pH 9-10 the solution was evaporated to dryness, taken up in a small volume of water (3-5 ml.), filtered and dried in a tared vessel.

Sodium isobutyrate-1-C^{14}: Specific Activity, 1.72 μc/mg. 97.5% yield.

Sodium isovalerate-1-C^{14}: Specific Activity, 3.35 μc/mg. 96.0% yield.

Sodium isocaproate-1-C^{14}: Specific Activity, 1.70 μc/mg. 95.5% yield.

Isoamyl Bromide-1-C^{14}. -- Sodium isovalerate-1-C^{14} (2.10 g., 16.9 mmoles, 7.09 mc. total activity) was added to an ignition tube containing 5.40 g. cetyl bromide (17.7 mmoles). The tube was sealed and heated with shaking at 280°C for eleven hours. A high pressure hydrogenation bomb (4) was used

(4) Micro bomb. American Instrument Co., Silver Springs, Maryland.

for this step but no pressure was placed in the bomb. After reaction the ignition tube contents were dissolved in ether and filtered. An ether-insoluble, water-soluble residue containing 0.05 mc. was left on the filter paper. The filtrate was washed into a 200 ml. hydrogenation bomb and warmed to 40-50°C to evaporate most of the ether. Then, 5.5 g. of copper chromite as catalyst (5) was added and the bomb closed, attached to a vacuum

(5) See H. Adkins in "Organic Synthesis, Vol. II", John Wiley and Sons, Inc., New York (1943), p. 144.

and pumped one and one-half hours.

The bomb was pressured to 2550 p.s.i. with hydrogen and heated at 250°C with shaking for ten hours. The hydrogen was released through a spiral trap equipped with a sintered disk (6) and cooled with liquid air. The bomb was

(6) B.M. Tolbert, et.al., J.Org.Chem., 14, 527 (1949)

then pumped overnight while warmed at 80-85°C.

The alcohol thus obtained was converted to the bromide using phosphorus tribromide (6) and purified by washing with water and drying over phosphorus pentoxide. Yield 1.88 g., or 73% based on the isovalerate used.

Isocaproic Acid-2-C^{14}. -- The isoamyl bromide (1.88 g., 12.4 mmoles) was distilled into a vessel containing 1.65 g. potassium cyanide (25 mmoles) and 0.2 g. potassium iodide in 25 ml. of ethanol. This reaction mixture was refluxed 42 hours, at which time it was cooled and 4 g. silver sulfate and 30 ml. water added. A distillation head and condenser was attached and the labeled nitrile slowly distilled out.

To the distillate 15 g. of potassium hydroxide was added and the solution was refluxed for 24 hours. The mixture was acidified with 50 ml. of 10 N sulfuric acid and the acid steam distilled, to give 0.949 g. of sodium caproate-2-C^{14} with a specific activity of 2.90 ± 0.1 μc/mg. (calculated 3.01 ± 0.1 μc/mg.). Yield 55% based on isoamyl bromide used.

Leucine-2-C^{14}. -- (a) Bromination of isocaproic acid-2-C^{14}: Sodium isocaproate-2-C^{14} (0.508 g., sp. act. 2.90 μc/mg., 1.48 mc.) was placed in a Pyrex tube which was connected to a spiral trap (see Figure 1). The salt and equipment was then dried by pumping a vacuum of about 10 microns on the system for an hour. The system was then disconnected from the vacuum line and the trap immersed in a cooling bath at -20°C. Dry HCl was then passed slowly over the sodium isocaproate which was heated. The isocaproic

acid thus formed was condensed in the spiral trap. At the completion of this reaction the trap was reconnected to the vacuum line, and the other end of the trap was connected to a bromination vessel (Figure 1). The trap and the vessel were cooled with liquid air, evacuated and the stopcock connecting them to the vacuum line closed. Then the free acid was distilled into the bromination vessel by heating the trap to 90-95°.

Inactive isocaproic acid, generated in the same manner from 0.375 g. sodium isocaproate and 0.2 ml. phosphorus tribromide was added. After standing overnight 0.04 g. red phosphorus and 0.02 g. iodine were added and the condenser fitted with a protecting Drierite tube connected to the vessel. Dry Ice was added to the condenser and the bromination flask heated on a steam bath for 30 minutes during which 2.3 ml. bromine was added dropwise.

The bromination flask was then cooled in liquid air and the condenser warmed with hot water. After everything had distilled into the bromination flask, 5 ml. of water was added to the vessel and the mixture slowly warmed to room temperature. Five more ml. water were added and the vessel was air swept for four hours to remove bromine. The product was then extracted with methylene chloride and the resulting solution pressure filtered; this was repeated several times to give a total volume of solution of about 25 ml.

(b) Amination of the α-Bromoisocaproic acid-2-C^{14}: The clean methylene chloride solution was transferred to an ignition tube and evaporated to dryness on the steam bath; the last traces of solvent were removed by use of the water pump at room temperature. Then 10 ml. of liquid NH_3 were

condensed in the tube, the tube sealed and left at room temperature for 40 hours. The tube was then chilled, opened, and the excess NH_3 evaporated off.

The residue was dissolved in 6 N hydrochloric acid and evaporated to dryness. The residue was dissolved in 95% ethanol, filtered and ethylene oxide passed through the solution for 20 minutes. The precipitate was recrystallized three times from water, using a decolorizing charcoal the first two times.

The yield was 308.6 mg. Anal. Calcd for $C_6H_{13}NO_2$: N, 10.69; sp. act., 1.76 ± 0.1 μc/mg. Found: N, 10.55, 10.53; sp. act. 1.85 ± 0.1 μc/mg. From the mother liquors an additional 121.4 mg. was obtained; sp. act. 1.8 μc/mg. Total yield 52% based on isocaproic acid or 20.3% based on barium carbonate used.

DL-Leucine-1-C^{14}. -- By the procedure just described sodium isocaproate-1-C^{14} (1.93 g., 1.70 ± 0.1 μc/mg) was converted to DL-leucine-1-C^{14} (790 mg., sp. act., 1.71 ± 0.1 μc/mg. The yield was 42% based on isocaproate used or 40% based on starting barium carbonate.

SUMMARY

The following compounds have been synthesized labeled with C^{14}. The yields, based on $C^{14}O_2$ used, are indicated in parentheses: Sodium isobutyrate-1-C^{14} (97.5); sodium isovalerate-1-C^{14} (96.0); sodium isocaproate-1-C^{14} (95.5); sodium isocaproate-2-C^{14} (39); DL-leucine-1-C^{14} (43.5) and DL-leucine-2-C^{14} (29.3). A novel method was developed for the preparation of alcohols from intermediate weight fatty acids and by this method isoamyl bromide-1-C^{14} was prepared in 73% yield based on sodium isovalerate-1-C^{14} used.

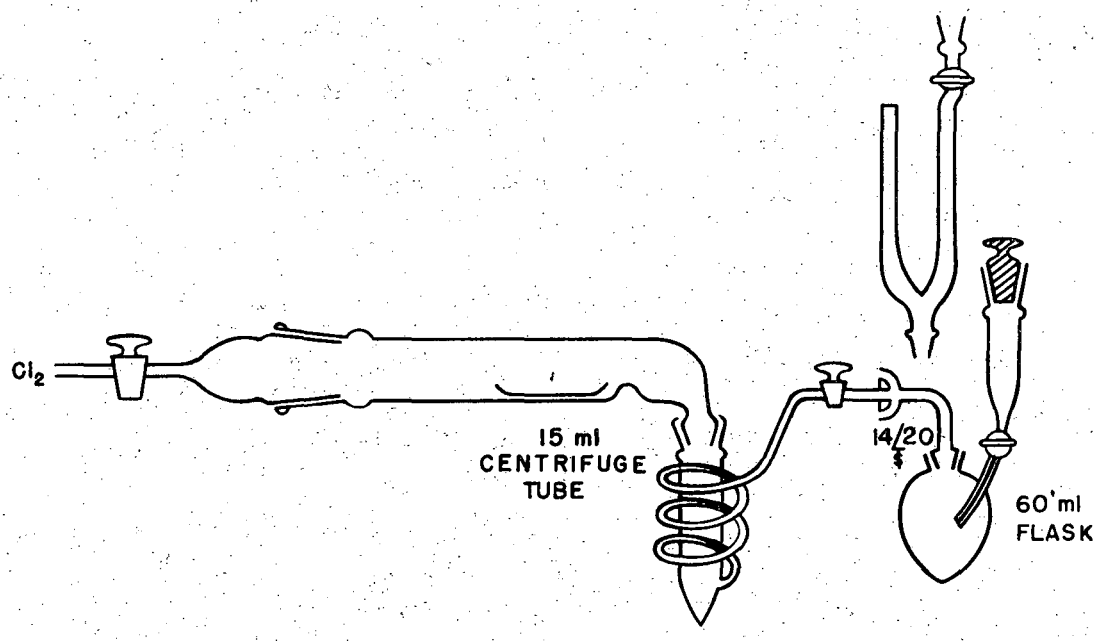

FIG. 1

ACID GENERATION UNIT & BROMINATION VESSEL WITH CONDENSER SEPARATED.