SYNTHESIS OF SHORT CHAIN CARBOXYLIC ACIDS LABELLED WITH ^{13}C AND ^{2}H AT VARIOUS POSITIONS

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SUMMARY

2-Methylpropionic, n-butyric, 2-methylbutyric and n-heptanoic acids labelled with ^{13}C or ^{2}H at various positions were synthesized. Carbon-13 was introduced to the carboxyl group by Grignard synthesis. Malonate ester synthesis was utilized to introduce labelled methyl group(s) at 2-position of the branched acids. Procedures for small scale synthesis by these commonly used methods are described in detail. In addition, a novel method for the introduction of a deuterium at the 2-position of these carboxylic acids by decarboxylation of the corresponding carboxyl-deuterated malonic acid is described.

Key Words: Carbon-13, Deuterium, Short Chain Carboxylic Acids

INTRODUCTION

For our studies on the metabolism of branched chain amino acids, we required 2-methylbutyrates and 2-methylpropionates labelled with ^{2}H and ^{13}C at various positions (1). Although a variety of methods are suitable for synthesizing short-chain carboxylic acids (2), we chose malonate ester synthesis (3) as a means of introducing stable isotope labels to hydrocarbon chains and Grignard synthesis (2) to introduce ^{13}C into carboxylic carbons. The commercial availability at relatively

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low cost of labelled alkylhalides and $^{13}CO_2$ makes these approaches particularly attractive.

Malonate ester and Grignard syntheses are commonly used methods for the synthesis of carboxylic acids, but details in the literature are incomplete regarding their practical application to the preparation of ^{13}C and ^{2}H labelled compounds. We describe here in detail the small scale syntheses of 2-methylpropionic-1-^{13}C, 2-methyl-^{13}C-propionic-3-^{13}C, 2-methyl-$^{2}H_3$-propionic-3,3,3-$^{2}H_3$, and 2-methylpropionic-3,3,3-$^{2}H_3$ acids. Also included are 2-methyl-^{13}C-butyric, 2-methyl-$^{2}H_3$-butyric and 2-methylbutyric-1-^{13}C acids.

In addition, a novel method for the introduction of deuterium at the 2-position <u>via</u> decarboxylation of malonic acids is described in this report, with data from the syntheses of 2-methylbutyric-2-^{2}H, butyric-2-^{2}H, and heptanoic-2-^{2}H acids. In comparison with other methods, this technique has the advantages of shorter reaction time, simpler procedure, smaller amounts of deuterium precursors such as $^{2}H_2O$ required and is specific for mono-deuteration.

RESULTS AND DISCUSSION

The synthetic schemes are summarized in the following reaction sequences:

I. Synthesis of 2-methyl-^{13}C-propionic-3-^{13}C and 2-methyl-$^{2}H_3$-propionic-3,3,3-$^{2}H_3$ acids via dialkylation of malonic esters

$$CH_2(COOEt)_2 \xrightarrow[2.\ 2\,RI]{1.\ NaOEt,\ EtOH} R_2C(COOEt)_2 \xrightarrow[2.\ H^+\\ 3.\ \Delta]{1.\ KOH,\ \Delta} R\text{-}\underset{\displaystyle R}{\underset{|}{CH}}\text{-}COOH$$

$$R: C^2H_3 \text{ or } {}^{13}CH_3$$

II. Synthesis of 2-methylpropionic-3,3,3-$^{2}H_3$, 2-methyl-$^{2}H_3$-butyric, and 2-methyl-^{13}C-butyric acids via monoalkylation of malonic esters.

$$RCH(COOEt)_2 \xrightarrow[2.\ R'I]{1.\ NaOEt,\ EtOH} R\text{-}\overset{R'}{\underset{|}{C}}(COOEt)_2 \xrightarrow[2.\ H^+\\ 3.\ \Delta]{1.\ KOH,\ \Delta} R\text{-}\overset{R'}{\underset{|}{C}}H\text{-}COOH$$

R: CH_3 *or* CH_3CH_2
R': $^{13}CH_3I$ *or* C^2H_3I

III. Grignard synthesis of 2-methylpropionic, 2-methylbutyric and butyric acids labelled at the carboxyl group.

$$R\text{-}\overset{R'}{\underset{|}{C}}H\text{-}Br \xrightarrow[2.\ ^{13}CO_2\\ 3.\ H^+]{1.\ Mg,\ Et_2O,\ \Delta} R\text{-}\overset{R'}{\underset{|}{C}}H\text{-}^{13}COOH$$

R: CH_3 *or* CH_3CH_2
R': CH_3 *or* H

IV. Synthesis of alkyl-2-^{2}H carboxylic acids.

$$R\text{-}\overset{R'}{\underset{|}{C}}(COOK)_2 \xrightarrow{^{2}H^+,\ ^{2}H_2O} R\text{-}\overset{R'}{\underset{|}{C}}(COO^{2}H)_2 \xrightarrow[2.\ H_2O]{1.\ \Delta} R\text{-}\overset{R'}{\underset{|}{C}}{}^{2}H\text{-}COOH$$

R and R' vary depending on the desired product

The malonate ester synthesis of 2-methylbutyrates and 2-methylpropionates followed parallel procedures except for the decarboxylation step. Ethanol and sodium ethoxide (prepared in situ) were effective as solvent and base, respectively, although other choices are available (e.g., benzene-Na; toluene-K) (3). In our experiments, we did dialkylation of unsubstituted malonate or monoalkylation of mono-substituted malonate. Since we did not have to be concerned with excessive alkylation we chose the most reactive halide, the iodide. In the synthesis of straight chain acids via mono-alkylation of malonate, it is possible to prevent

or limit dialkylation by taking suitable precautions (e.g., large solvent excess, less reactive halides) (3). To maximize our yields and purity we used a slight excess of the alkyl iodide and sodium ethoxide. In several preliminary experiments with natural abundance compounds we observed that the reaction was much more complete with the excess amounts of the reagents.

We did notice that saponification was considerably easier to effect for diethyl 2-ethyl-2-methylmalonate than for diethyl 2,2-dimethylmalonate. This is reflected in the amount of time required for saponification. The hydrolysis of the former required only 12 hours of heating under reflux in 12.5 N KOH in H_2O, whereas that of the latter took 24 hours under the same conditions. It is well known that the ease of saponification of malonate esters is dependent upon the structure, yet the observed difference was much more profound than expected for such similar compounds.

The decarboxylation of 2-ethyl-2-methylmalonic acid proceeded efficiently by refluxing in aqueous sulfuric acid. In contrast, we were unable to completely decarboxylate 2,2-dimethylmalonic acid in aqueous solution even with a large amount of sulfuric acid and long reflux times (five days). We pyrolyzed the crystals of the 2,2-dimethylmalonic acid at 195°C to accomplish decarboxylation. This is in accord with the observations of Norris (4) who reported a decomposition temperature for dimethylmalonic acid considerably higher than for structurally similar dialkylated malonic acids.

In the Grignard syntheses a closed system was necessary to make effective use of the $^{13}CO_2$. To accomplish this we froze the Grignard reagent in the reaction flask with liquid nitrogen and evacuated the system prior to the introduction of $^{13}CO_2$ as discussed in EXPERIMENTAL.

Several methods are available for introducing deuterium into the 2-position of carboxylic acids. The method described by Atkinson, Csakvary, Herbert and Stuart (5) requires prolonged (for 24 hours) and repeated (5 times) heating at 150°C of a 2.5 M solution of the potassium or sodium salt in ^2H_2O in the presence of a small amount of sodium or potassium deuteroxide. A large excess of deuterium oxide is necessary. Caspar, Greff and Wolff (6) proposed refluxing the acid as

the methyl ester with CH_3ONa in a CH_3O^2H/HMPT (hexamethyl phosphoric triamide) solution. The reaction times for this method are shorter but the percent incorporation of deuterium is lower than that obtained by the former method. These two methods are not specific for monodeuteration of acetic and mono-substituted acetic acid.

It has been suggested that deuterium quenching of the 2-metalated (lithiated) aliphatic acids is a valuable method for introducing one or two deuteriums in the 2-position (7). However, the effectiveness of incorporation appears to be variable for different acids.

We describe here an alternative method for the synthesis of 2-deuterated carboxylic acids based upon the decarboxylation of carboxyldeuterated malonic acids as illustrated in Scheme IV. The reaction mechanism can probably be expressed by the following equation in analogy to that for the decarboxylation of carboxyldeuterated 3-keto acids (8) (Fig. 1).

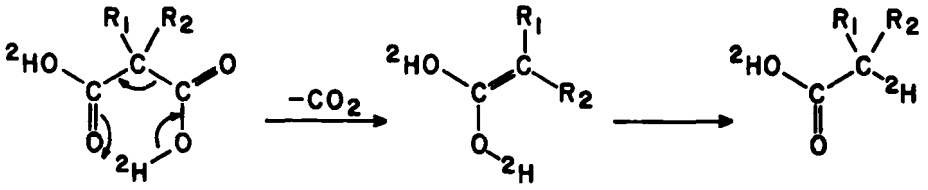

Fig. 1. Mechanism of decarboxylation of carboxyldeuterated malonate to produce 2-deuterated carboxylic acids.

The advantage of this method can be summarized as 1) short reaction time for deuterium incorporation, 2) economical use of deuterated precursors, 3) easy recovery of products and 4) good percent deuterium enrichment. However, the utility of this reaction is limited by the availability of malonic acid precursors and the percent incorporation of deuterium by this method is somewhat lower than those obtained by the method of Atkinson, et al. (5).

EXPERIMENTAL

Materials---The materials labelled with stable isotopes were procured from the following companies. Methyl-$^{2}H_3$ iodide (99+ atom % ^{2}H), water-$^{2}H_2$ (99.5 atom % ^{2}H), and 98% sulfuric acid-$^{2}H_2$ (99.5 atom % ^{2}H) in $^{2}H_2O$ were from Aldrich Chemical Corp., Milwaukee, WI. Methyl-^{13}C iodide (90+ atom % ^{13}C) and carbon-^{13}C dioxide (90 atom %^{13}C) were from Merck, Sharpe and Dohme, Montreal, Canada.

Analysis of products---Chemical purity of all of the compounds was analyzed by gas chromatography both as free acid and as methyl ester using 25% neopentylglycol adipate - 2% phosphoric acid and 10% OV-11 columns, respectively. The structures and isotope enrichment of all products were verified by both gas chromatograph-mass spectrometry (GC/MS) as well as by proton nuclear magnetic resonance (NMR).

GC/MS was performed on a Varian MAT 111 gas chromatograph-mass spectrometer using a 10% OV-11 column as the inlet column. The acids were analyzed as methyl esters. The molecular ion or $(M-15)^{+}$ ion was analyzed for isotopic enrichment in the 2-methylpropionates and 2-methylbutyrates, respectively. Appropriate fragment ions were used for analysis of isotopic enrichment in the other compounds synthesized. The column temperature was 50°C; the injector temperature 250°C; separator temperature 300°C; inlet line temperature 280°C and the ion source temperature 250°C. The ionizing voltage was 80 eV.

Nuclear magnetic resonance was performed on the free acids in solution using either a Bruker HX-270 or a Varian T-60 NMR spectrometer. Typical concentration of the samples was 30 mg per ml $^{2}H_2O$. Comparison with corresponding natural abundance compounds and analysis of coupling due to the ^{13}C and ^{2}H in each case helped to prove the structure and to measure the isotope enrichment. Isotope enrichment determined by GC/MS and by NMR agreed to within 5%.

Sodium 2-methyl-^{13}C-butyrate (1)---Clean sodium (0.0213 mol) was placed in a 25 ml, argon flushed, round-bottom flask equipped with a magnetic stirring bar, reflux condenser connected to a mineral oil bubbler and an addition funnel. Slow argon gas flow was maintained throughout the reaction. Ethanol (4 ml) previously

distilled over sodium was placed in the addition funnel. 1.5 ml of ethanol was added to the sodium with stirring at room temperature. After reflux, which occurred during the addition, had subsided, a heating mantle was used to raise the reaction temperature until the sodium melted (98°C). The remainder of the ethanol was added dropwise and reflux was continued. After one hour, all the metal was consumed and a yellow solution resulted with some white precipitate.

The sodium ethoxide solution was cooled until reflux subsided and diethyl 2-ethylmalonate (0.0172 mol) was added dropwise to the sodium ethoxide with constant stirring. The mixture was then stirred vigorously with mild heating until all the precipitate dissolved. These conditions were continued for one hour to maximize formation of the malonate ester enolate. Methyl-^{13}C iodide (0.0210 mol) was added very slowly to the reaction while heating was interrputed. A milky white solution resulted. Reflux was run for three hours after complete addition.

Heating and argon flow were stopped and the solution cooled to room temperature with stirring. The reaction mixture was washed with 10 ml of water to extract ethanol. The upper layer was pipetted off and placed into a round-bottom flask with a reflux condenser. A cold solution of potassium hydroxide (0.125 mol) in 10 ml of water was added and the mixture was vigorously refluxed for twelve hours with stirring.

Sulfuric acid (0.0828 mol) was added dropwise to the ice-cooled reaction flask with stirring. The acidified solution was refluxed for three hours to effect decarboxylation of the disubstituted malonic acid. The cooled solution was extracted six times with equal volumes of diethyl ether. The organic extracts were dried over anhydrous sodium sulfate, filtered, solvent evaporated under reduced pressure and the 2-methyl-^{13}C-butyric acid (b.p. 177°C) distilled in a mini-distillation apparatus between 120° and 150°C. The distillate was titrated to pH 9.0 with 1.0 N sodium hydroxide and the resulting salt solution lyophilized to yield the product. The chemical purity, isotope enrichment and yield were 99, 90 and 63%, respectively.

Sodium 2-methyl-$^{2}H_3$-butyrate (2)---This deuterated compound was prepared in the same manner as compound 1. Trideuteromethyl iodide was utilized as the

alkylating agent. The chemical purity, isotope enrichment and yield of the product were 98, 99 and 57%, respectively.

Sodium 2-methyl-^{13}C-propionate-3-^{13}C (3)---The sodium ethoxide solution was prepared from 0.0415 mol of sodium and 10 ml of ethanol and the sodium enolate prepared from malonate diethyl ester (0.0187 mol) both as described for compound 1. Dialkylation was achieved by adding 0.0420 mol of $^{13}CH_3I$. Reflux was continued for six hours.

After washing with 10 ml of water, the ester layer was pipetted off and refluxed with potassium hydroxide (0.1363 mol) in 12 ml water for twenty-four hours to complete saponification. The cooled solution was acidified to pH 1 and extracted with diethyl ether. The organic extracts were dried over anhydrous sodium sulfate, filtered, and the ether removed on a rotary evaporator. 2,2-Dimethyl-^{13}C-malonic acid was recrystallized from ether by addition of hexane. The recovered acid was dried at 60°C.

The acid crystals were placed in a 25 ml round-bottom flask with a magnetic stirrer and reflux condenser attached to a mineral oil bubbler. The flask was heated by an oil bath. The temperature was raised slowly to 195°C at which point decarboxylation started. After evolution of carbon dioxide ceased, the flask was kept immersed in the oil bath at 180°C for one hour. The resulting 2-methyl-^{13}C-propionic -3-^{13}C acid was distilled and titrated to pH 9.0 with 1.0 N sodium hydroxide (aqueous). The salt was lyophilized to yield the product. The chemical purity, and yield were 99 and 44%, respectively. The isotope enrichment was 90% at each methyl group.

Sodium 2-methyl-$^{2}H_3$-propionate-3,3,3-$^{2}H_3$ (4)---This deuterated acid was prepared in the same manner as compound 3. Trideuteromethyl iodide was utilized as the alkylating agent. The chemical purity, isotope enrichment and yield were 99, 99 and 34%, respectively.

Sodium 2-methylpropionate-3,3,3-$^{2}H_3$ (5)---This deuterated acid was prepared in a manner similar to the synthesis of compound 3, except that the starting material was diethyl 2-methylmalonate. The ratio of the amounts of sodium,

malonate ester and methyl-$^{2}H_3$ iodide was 1.11:1.00:1.11. The chemical purity, isotope enrichment and yield were 97, 99 and 29%, respectively.

Sodium 2-methylbutyrate-2-^{2}H (6)---2-Ethyl-2-methylmalonic acid was prepared from diethyl 2-ethylmalonate in the same manner as compound 1, alkylating with CH_3I. The disubstituted malonic acid was converted to the dipotassium salt. The salt was dried under reduced pressure (1.0 mm Hg). Ten grams of the salt was dissolved in 15 ml of deuterium oxide and acidified with $^{2}H_2SO_4$ to pH 1.0. The solution was extracted with diethyl ether, dried over anhydrous sodium sulfate, filtered and recrystallized from ether by addition of hexane. The recovered crystals were dried under vacuum at room temperature for 24 hr. The acid was placed in a 50 ml round-bottom flask fitted with reflux condenser connected to a mineral oil bubbler. The acid was pyrolyzed in the manner already described for compound 3. The distilled acid was titrated with sodium hydroxide and lyophilized to yield the product. The chemical purity and isotope enrichment were 98 and 80%, respectively. Although it was not determined accurately, the yield was high as expected from the simple procedure.

Sodium 2-methylpropionate-2-^{2}H (7)---This deuterated acid was prepared by the method of Atkinson, et al. (5). The chemical purity, isotope enrichment, and yield were 99, 73 and 54%, respectively.

Sodium butyrate-2-^{2}H (8)---This acid was prepared from ethylmalonic acid in the same manner as compound 6. The chemical purity and isotope enrichment were 99 and 80%, respectively. The yield was not determined.

Sodium heptanoate-2-^{2}H (9)---This acid was prepared from n-pentylmalonic acid in the same manner as compound 6. The chemical purity and isotope enrichment were 93 and 61%, respectively. The yield was not determined.

Sodium 2-methylbutyrate-1-^{13}C (10)---This compound was prepared by Grignard reaction of sec-butylmagnesium bromide with $^{13}CO_2$ according to the method of Marshall, et al. (9). To adapt this for our purposes, we froze the Grignard reagent in a one-half litre double-necked flask at liquid nitrogen temperature. A

sealed one litre glass ampoule of $^{13}CO_2$ was attached by a short rubber tube to a stopcock that was fitted on to one neck of the flask. The entire system was evacuated to 1.0 mm Hg. The stopcock leading to the pump was closed and the seal on the ampoule was broken. The $^{13}CO_2$ expanded into the reaction flask. The ampoule was warmed slightly by hand to force most of the gas into the reaction vessel. After ten minutes the stopcock leading to the ampoule was closed. The reaction vessel was transferred to a Dry Ice/isopropyl alcohol bath where it remained for twenty minutes. The reaction mixture was then stirred in an ice bath for one hour.

The flask was opened to the atmosphere and diluted with water at 0°C, acidified with 6.0 N HCl, and the organic layer removed. The water layer was extracted several times with diethyl ether and all the organic layers were combined and dried over anhydrous sodium sulfate. The acid was isolated in the same manner as compound 1. The chemical purity, isotope enrichment and yield were 99, 92 and 82%, respectively.

Sodium 2-methylpropionate-1-^{13}C (11)---This compound was prepared in the same manner as compound 10 from the reaction of isopropylmagnesium bromide with $^{13}CO_2$. The chemical purity, isotope enrichment and yield were 99, 90 and 74%, respectively.

Sodium butyrate-1-^{13}C (12)---This compound was prepared in the same manner as compound 10, from the reaction of propylmagnesium bromide with $^{13}CO_2$. The chemical purity, isotope enrichment and yield were 99, 92 and 81%, respectively.

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