Synthesis of carbon-13-labeled tetradecanoic acids

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Summary The synthesis of tetradecanoic acid enriched with ^{13}C at carbons 1, 3, or 6 is described. The label at the carbonyl carbon was introduced by treating 1-bromotridecane with $K^{13}CN$ (90% enriched) to form the ^{13}C-labeled nitrile, which upon hydrolysis yielded the desired acid. The [3-^{13}C]tetradecanoic acid was synthesized by alkylation of diethyl sodio-malonate with [1-^{13}C]1-bromododecane; the acid was obtained upon saponification and decarboxylation. The label at the 6 position was introduced by coupling the appropriately labeled alkylcadmium chloride with the half acid chloride methyl ester of the appropriate dioic acid, giving the corresponding oxo fatty acid ester. Formation of the tosylhydrazone of the oxo-ester followed by reduction with sodium cyanoborohydride gave the labeled methyl tetradecanoate which, upon hydrolysis, yielded the desired tetradecanoic acid. All tetradecanoic acids were identical to unlabeled analogs as evaluated by gas–liquid chromatography and infrared or NMR spectroscopy. These labeled fatty acids were used subsequently to prepare the correspondingly labeled diacyl phosphatidylcholines.—**Sparrow, J. T., K. M. Patel, and J. D. Morrisett.** Synthesis of carbon-13-labeled tetradecanoic acids. *J. Lipid Res.* 1983. **24:** 938–941.

Supplementary key words nuclear magnetic resonance • dimyristoylphosphatidylcholine

Fatty acids labeled with isotopes such as ^{14}C, ^{13}C, ^{2}H, and ^{3}H are in increasing demand for biological studies. Compounds enriched in the stable isotopes ^{2}H and ^{13}C are of particular importance for deuterium and carbon magnetic resonance experiments in biological systems. However, reports of synthetic ^{13}C-labeled fatty acids (1–3) are limited compared to ^{2}H-labeled fatty acids (1, 4). Our studies of apolipoprotein-phospholipid interaction have required dimyristoylphosphatidylcholine (DMPC), ^{13}C-enriched at specific carbons. To our knowledge, only a few ^{13}C(1-^{13}C, 2-^{13}C, and 7-^{13}C)-labeled tetradecanoic acids have been reported in the literature (2, 3). Accordingly, we have focused our attention on improving the yields of reactions used to obtain these labeled compounds. We have found that the synthesis of labeled long chain nitriles can be greatly improved using 18-crown-6 as a catalyst. This catalyst is also very useful for the rapid saponification of fatty acid methyl esters. We have previously reported (3) improved yields of methyl ketones by using methylcadmium chloride instead of the more commonly used dimethylcadmium (5, 6). From the yield in the present and other syntheses, we believe that freshly prepared alkylcadmium chlorides lead to higher yields of oxo-esters than do dialkylcadmiums (6). We have successfully applied, for the first time, the procedure of Hutchings, Maryanoff, and Milewski (7) to the reduction of oxo-esters to fatty acid esters. The use of column chromatography instead of distillation of the products also contributed to the ease of isolation and consequently helped to increase the yield. We report here the results of these improved reaction conditions for the syntheses of [1-^{13}C]-, [3-^{13}C]-, and [6-^{13}C]-labeled tetradecanoic acids (myristic acids). These reaction conditions have also been used to synthesize other labeled fatty acids in high yield.

Abbreviations: NMR, nuclear magnetic resonance; TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

RESULTS AND DISCUSSION

Fatty acids labeled in the carboxyl group are usually obtained either by treatment of the appropriate Grignard reagent with $^{13}CO_2$ (8, 9) or by reaction of $Na^{13}CN$ and the alkyl halide followed by hydrolysis. Verderas et al. (10) reported the synthesis of [1-^{13}C]tetradecanoic acid from the nitrile which was prepared by stirring 1-bromotridecane, and $Na^{13}CN$ in dimethylsulfoxide for 4 days. We have utilized 18-crown-6 to catalyze the reaction of 1-bromotridecane and $K^{13}CN$ (90% enriched) in acetonitrile and have obtained an excellent yield of the ^{13}C nitrile in 4 hr. The labeled nitrile was converted to methyl [1-^{13}C]tetradecanoate by methanolic hydrogen chloride. We have used 18-crown-6 to catalyze the KOH saponification (11) of the methyl ester to potassium [1-^{13}C]tetradecanoate, which upon acidification yielded [1-^{13}C]tetradecanoic acid in excellent yield.

[3-^{13}C]Tetradecanoic acid was prepared via a malonic ester synthesis utilizing [1-^{13}C]1-bromododecane to obtain diethyl n-dodecylmalonate. Upon saponification and decarboxylation, the desired [3-^{13}C]tetradecanoic acid was obtained in excellent yield. The required [1-^{13}C]1-bromododecane was synthesized as follows. 1-Bromoundecane was treated with $K^{13}CN$ in the presence of 18-crown-6 to form the nitrile which, upon hydrolysis, yielded methyl [1-^{13}C]dodecanoate. This compound was reduced to [1-^{13}C]dodecanol with bis(2-methoxyethoxy)-aluminum hydride in benzene. [1-^{13}C]Dodecanol was treated with 48% HBr to obtain [1-^{13}C]1-bromododecane.

[6-^{13}C]Tetradecanoic acid was synthesized from [1-^{13}C]1-bromononane, which in turn was synthesized

from 1-bromooctane by the reaction sequence described above. Adapting the improvements to the alkylcadmium coupling reported by Patel, Morrisett, and Sparrow (3), [1-^{13}C]nonyl magnesium bromide was generated in ether and converted to [1-^{13}C]-nonylcadmium chloride by adding an equimolar amount of anhydrous cadmium chloride. After replacing the ether with benzene, an equivalent of methyl 4-(chloroformyl)-butyrate was added and the reaction was stirred for 24 hr. After workup, methyl [6-^{13}C]5-oxotetradecanoate was obtained in excellent yield. Reduction of methyl [6-^{13}C]5-oxotetradecanoate was accomplished by *p*-toluenesulfonylhydrazide and sodium cyanoborohydride (7) to obtain methyl [6-^{13}C]tetradecanoate. Hydrolysis with potassium hydroxide/18-crown-6 yielded [6-^{13}C]tetradecanoic acid.

The labeled fatty acids were converted to the corresponding anhydrides with N,N′-dicyclohexyl carbodiimide and reacted with the cadmium chloride complex of glycerophosphorylcholine in the presence of 4-pyrrolidinopyridine to obtain the appropriately labeled dimyristoylphosphatidylcholine (12).

EXPERIMENTAL MATERIALS

Bromooctane, bromoundecane, bromotridecane, bis(2-methoxyethoxy) aluminum hydride (70% solution in benzene, RedAl), 18-crown-6, diethyl malonate, and methyl 4-(chloroformyl)-butyrate were all obtained from Aldrich Chemical Co. (Milwaukee, WI). K^{13}CN (90% ^{13}C) and [^{13}C]iodomethane (90% ^{13}C) were from KOR Isotopes (Cambridge, MA). Sodium cyanoborohydride and anhydrous cadmium chloride were from Ventron (Danvers, MA). Silica gel G-60 was from Brinkman. All solvents were purchased from Burdick and Jackson (Muskegon, MI). GLC analysis was performed on a Hewlett Packard model 5830A GC with a 1-m column of 10% SP-2330 on Supelcoport. ^{13}C NMR spectra were recorded on a Varian XL-100-15 spectrometer. The chemical shift for the intense signal of the labeled carbon is reported in parts per million from an external capillary of tetramethylsilane. Infrared spectra were recorded on a Beckman Accu-Lab 4.

Synthesis of [1-^{13}C]tetradecanoic acid

[*^{13}CN*]*Tridecylnitrile.* Bromotridecane (2.04 g, 7.7 mmole), K^{13}CN (90% ^{13}C), (0.5 g, 7.7 mmol) and 18-crown-6 (2.03 g, 7.7 mmol) were placed in a 100-ml round-bottom flask and 30 ml of acetonitrile was added. The reaction mixture was refluxed for 4 hr, then diluted with water (50 ml), and the product was extracted with 3 × 50 ml of hexane. The hexane was dried over anhydrous magnesium sulfate and removed by rotary evaporation under reduced pressure. The residual oil was purified on a silica gel column (1 × 10 cm) equilibrated with hexane. The product eluted with hexane–ethyl acetate 9:1. Yield: 1.56 g (97%); IR, 2215 cm^{-1} (-C≡N); NMR, 119.5 ppm (-C≡N). GLC analysis indicated the product had the same retention time as unlabeled tridecylnitrile.

Methyl [1-^{13}C]Tetradecanoate. [^{13}CN]Tridecylnitrile (1.56 g, 7.5 mmol) was added to 50 ml of methanolic hydrogen chloride (30% w/w) in a 200-ml round-bottom flask and stirred for 4 hr. Cold water (50 ml) was added and the product was extracted with diethyl ether (3 × 25 ml). The ether extracts were dried over magnesium sulfate and the ether was evaporated. The residue was purified on a silica gel column (1 × 10 cm) equilibrated with hexane. The product eluted with hexane–ethyl acetate 8:2. Yield: 1.75 gm (96%); mp 19–20°C, lit (13) mp 19°C; IR, 1715 cm^{-1} (-C=O ester).

[1-^{13}C]Tetradecanoic acid. Methyl [1-^{13}C]tetradecanoate (1.65 gm, 6.58 mmol), powdered potassium hydroxide (0.4 g, 7.4 mmol), and 18-crown-6 (0.4 g, 1.5 mmol) were mixed in 20 ml of dry benzene in a 100-ml flask and stirred for 12 hr at room temperature. The reaction mixture was acidified with 6 N hydrochloric acid and extracted with 3 × 25 ml of benzene. After drying over anhydrous sodium sulfate, the benzene was removed under reduced pressure to yield the desired tetradecanoic acid. Yield: 1.45 g, (96%); mp 54–55°C, lit (13) mp 55°C; IR 1730 cm^{-1} (-C=O acid); NMR, 176.25 ppm (COOH). The GLC retention time and the ^{1}H NMR spectra were identical with those of authentic tetradecanoic acid.

Synthesis of [3-^{13}C]Tetradecanoic acid

[*1-^{13}C*]*Dodecanol.* Methyl [1-^{13}C]dodecanoate was prepared in 90% yield from 1-bromoundecane by the procedure described above for methyl [1-^{13}C]-tetradecanoate. Methyl [1-^{13}C]dodecanoate (3.0 g, 13.9 mmol) in 10 ml of dry benzene was added to RedA1 (70% solution of bis-(2-methoxyethoxy) aluminum hydride in benzene) under N_2 over 5 min. The reaction mixture was then stirred for 5 hr. The minimum amount of cold water required to destroy excess reagent was carefully added until the evolution of hydrogen was no longer observed. The benzene layer was removed and the aqueous layer was extracted with benzene (3 × 25 ml). The combined benzene extracts were dried over magnesium sulfate and the benzene was removed in vacuo. The residual oil was purified on a silica gel column (1 × 10 cm). The [1-^{13}C]dodecanol was eluted with hexane–ethyl acetate 8.5:1.5. Yield: 2.5 g (95%). TLC, GLC, and ^{1}H NMR properties of the [1-^{13}C]dodecanol were identical to those of unlabeled dodecanol.

[*1-^{13}C*]*1-Bromododecane.* [1-^{13}C]Dodecanol (2.5 g, 13.4

mmol) was refluxed with 8 ml of 48% HBr and 0.3 ml of conc. H_2SO_4 for 5 hr. Cold water (25 ml) was slowly added and the [1-^{13}C]1-bromododecane was extracted with 3 × 25 ml of hexane. The hexane layer was dried over anhydrous magnesium sulfate and removed under reduced pressure. The residual oil was purified on a silica gel column (1 × 10 cm) equilibrated with hexane. [1-^{13}C]1-Bromododecane was eluted with hexane. Yield: 3.2 g (95%). The IR spectrum indicated no -OH absorption at 3300 cm^{-1}. The GLC retention time and ^{1}H NMR chemical shifts of the product were identical to those of unlabeled 1-bromododecane.

Diethyl [3-^{13}C]n-dodecylmalonate. Diethyl malonate (2.4 g, 15 mmol) was added to a suspension of sodium hydride (0.63 g, 15 mmol, 57% dispersion in oil, Ventron) in N,N-dimethylformamide (DMF)–benzene 1:1 to form diethyl sodio-malonate. After 30 min, [1-^{13}C]1-bromododecane (3.1 g, 12.5 mmol) was added and the reaction mixture was stirred at room temperature overnight. After diluting the mixture with 25 ml of cold water, the product was extracted with 3 × 25 ml of diethyl ether. The ether extracts were dried over anhydrous magnesium sulfate and the ether was evaporated. The residue containing diethyl [3-^{13}C]n-dodecylmalonate and the excess diethyl malonate were separated on a silica gel column (1 × 20 cm) using stepwise elution with 10%, 20%, and 30% ethyl acetate in hexane; the product was eluted in 20% ethyl acetate. Evaporation of the solvent afforded 3.6 g (80% yield) of diethyl [3-^{13}C]n-dodecylmalonate. IR: 1715 cm^{-1}; NMR, 28.5 ppm. The GLC retention time was identical to that of an unlabeled sample.

[3-^{13}C]Tetradecanoic acid. Diethyl [3-^{13}C]n-dodecylmalonate (3.6 g, 11.8 mmol) was refluxed with 10 N H_2SO_4 for 24 hr and then heated at 180° for 30 min. The reaction mixture was cooled and carefully diluted with 25 ml of water. The product was extracted with 3 × 25 ml diethyl ether. The ether layer was dried over sodium sulfate and the solvent was removed. A residual solid was obtained that was crystallized from boiling hexane to yield 2.4 g (89%) of [3-^{13}C]tetradecanoic acid, mp 55°C. IR: 1730 cm^{-1} (C=O); NMR, 24.65 ppm. The GLC behavior was identical to that of authentic unlabeled material.

Synthesis of [6-^{13}C]tetradecanoic acid

[1-^{13}C]1-Bromononane. [1-^{13}C]1-Bromononane was synthesized from 1-bromooctane using the sequence of reactions and conditions described above for [1-^{13}C]1-bromododecane in overall 60% yield; NMR: 33.25 ppm. GLC and chromatographic behavior were identical to those of authentic unlabeled material.

Methyl [6-^{13}C]5-Oxotetradecanoate. [1-^{13}C]1-Bromononane (3.0 g, 14.5 mmol) in 15 ml of anhydrous diethyl ether was added to 0.35 g (0.015 g-atom) of magnesium turnings in 25 ml of anhydrous diethyl ether under N_2. The reaction started immediately and after 1 hr the metal was consumed. The reaction mixture was cooled to 4°C and powdered anhydrous cadmium chloride (2.67 g, 14.5 mmol) was added. The icebath was removed and after 1 hr at room temperature the diethyl ether was completely removed by distillation. Sixty ml of anhydrous benzene was added and 20 ml of benzene was distilled to ensure complete removal of the diethyl ether. The reaction mixture was then cooled to 0°C and methyl 4-(chloroformyl)-butyrate (2.38 g, 14.5 mmol) was added in 10 ml of benzene over a 5-min period. The reaction mixture was stirred overnight at room temperature. A 2% sulfuric acid solution was added until two distinct phases formed. The benzene layer was collected and dried over anhydrous magnesium sulfate. The benzene was removed under reduced pressure and the product was purified on a silica gel column (1 × 20 cm). The methyl [6-^{13}C]5-oxotetradecanoate was eluted with hexane–ethyl acetate 8:2. Yield: 3.4 g, (92%); IR, 1735 cm^{-1} (ester C=O); 1715 cm^{-1} (keto C=O).

Methyl [6-^{13}C]Tetradecanoate. Methyl [6-^{13}C]5-oxotetradecanoate was reacted with (2.8 g, 15 mmol) *p*-toluenesulfonylhydrazide in 40 ml of DMF–sulfolane 1:1 containing 100 mg of *p*-toluene sulfonic acid. After 1.5 hr, sodium cyanoborohydride (3.6 g, 50 mmol) was added and the reaction mixture was heated at 110°C for 7 hr. The reaction was diluted with 50 ml of water, extracted with 4 × 50 ml hexane and the hexane extracts were dried over anhydrous magnesium sulfate. After the hexane was removed under reduced pressure, the crude ester was purified on a silica gel column (1.5 × 20 cm). Methyl [6-^{13}C]tetradecanoate was eluted with hexane–ethyl acetate 9:1. Yield: 2.7 g (88%). The oil crystallized when stored at 4°C; mp, 19–20°C; IR, 1740 cm^{-1} (ester C=O), no keto C=O at 1715 cm^{-1}.

[6-^{13}C]Tetradecanoic acid. Methyl [6-^{13}C]tetradecanoate (2.6 g, 10.7 mmol) was hydrolyzed to [6-^{13}C]-tetradecanoic acid by the conditions used for [1-^{13}C]tetradecanoic acid to obtain 2.2 g (92%) of product; mp, 55°C; NMR, 29.35 ppm. GLC behavior and IR and ^{1}H NMR spectra were identical to those of unlabeled tetradecanoic acid.

We wish to thank Ms. Sarah Myers for editorial assistance, Mrs. Quein Pao for the GLC analysis, Dr. Roger Knapp for the ^{1}H and ^{13}C NMR spectra, and Mrs. Carol A. Coreathers for preparing the manuscript. J. T. Sparrow is an Established Investigator of the American Heart Association. This research was supported by a National Heart and Blood Vessel Research and Demonstration Center, Grant No. HL-17269, awarded to Baylor College of Medicine, and by a grant from the Robert A. Welch Foundation (Q-837).

Manuscript received 19 July 1982 and in revised form 11 March 1983.

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