SYNTHESIS OF [1,2,3,4,5-$^{13}C_5$] PALMITIC ACID (1)

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SUMMARY

Carbonylation of 1-undecene with ^{13}CO under a hydrogen atmosphere with palladium-stannous chloride catalyst afforded [1-13C]dodecanoic (lauric) acid on a 0.25 mole scale. Reduction to the alcohol and treatment with concentrated HBr gave 1-[1-^{13}C]bromododecane which was used to alkylate the Na^+, Li^+ mixed diananion of ethyl acetoacetate selectively at the 4-position in 73% yield. Reductive deoxygenation of the resulting 3-oxo intermediate, followed by saponification and purification, gave [1,2,3,4,5-$^{14}C_5$]hexadecanoic (palmitic) acid in 50% yield.

Key words: carbon-13, ^{13}CO, carbonylation, ethyl acetoacetate, lauric acid, palmitic acid, multiple labeling.

INTRODUCTION

Recent trends in the study of lipid metabolism by whole-body NMR (2,3) have prompted us to synthesize palmitic acid with multiple ^{13}C labels for use as a tracer in living tissues. Although [1-13C]fatty acids are commonplace commodities (4) and singly labeled skeletal chain analogs have been synthesized (5), the prospects for introducing multiple contiguous ^{13}C atoms into any target molecule are usually limited. Fortunately, DeGraw and co-workers (6) provided the point of departure for our efforts, having exploited ethyl [1,2,3,4-$^{13}C_4$]acetoacetate as a homologation synthon. In earlier work, Weiler (7) demonstrated the selective alkylation of methyl acetoacetate sodium/lithium

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dianion by a variety of alkyl halides at the 4-position in approximately 80% yields. This reaction has been corroborated recently by Roberts et al. (8) on the 100g scale, and we have applied it with equal success using uniformly ^{13}C-labeled ethyl acetatoacetate and 1-[1-^{13}C]bromododecane.

The latter was obtained from [1-^{13}C]dodecanoic acid after diborane reduction and bromination as described by Sparrow and co-workers (5). The carboxyl labeled fatty acid, in turn, was prepared by carbonylation of 1-undecene according to Knifton (9,10). Although unused heretofore in stable isotope chemistry, the Knifton reaction is intrinsically advantageous since it obviates the need for expensive [^{13}C]cyanide precursors and is amenable to molar scale fatty acid syntheses directly from ^{13}CO. Having secured ethyl 3-oxo-[1,2,3,4,5-$^{13}C_5$]hexadecanoate, we effected deoxygenation by reduction of the p-toluenesulfonylhydrazone with sodium cyanoborohydride (11). Saponification gave the desired product.

RESULTS AND DISCUSSION

A flow chart of synthetic events is shown in Scheme 1. According to the literature procedures (6), we prepared ethyl [1,2-$^{13}C_2$]acetate 1 in 82% yield by treating sodium acetate with diethylphosphite under reflux followed by fractional distillation. Self-condensation in cyclohexane catalyzed by KH gave the ethyl acetoacetate synthon 2 in 65% yield after distillation on an 0.3 mole scale. Preparation of the dodecyl synthon 6 also proceeded uneventfully according to the literature (9,10), but close attention was paid to Knifton's instructions in order to minimize by-product formation of 2-methyl-undecanoic acid during the reductive carbonylation of 1-undecene 3. Thus, [1-^{13}C]dodecanoic acid 4 was synthesized from $^{13}CO/H_2$ in 76% yield on an 0.5 mole scale (12). Diborane reduction according to Brown's usual method (13,14) and purification as described by Sparrow et al. (5) gave [1-^{13}C]dodecanol 5 in 93%

yield, with chromatographic properties identical to those of unlabeled dodecanol. Bromination (5) gave the [1-^{13}C]alkyl bromide 6 in 94% yield on an 0.1 mole scale.

Although short chain alkyl bromides and allylic bromides react completely and in high yield with the dianion of ethyl acetoacetate (7,8), we found its alkylation by [1-^{13}C]dodecylbromide to be more sluggish, affording at best a 60% conversion to the desired 3-oxo-fatty ester 7 despite several attempts to optimize the course of the reaction. The yield based on recovered starting materials was 73%. We observed no indications that alkylation had taken place at the undesired 2-position of the acetoacetate ester, so purification by vacuum distillation proved to be uncomplicated.

Deoxygenation to give ethyl palmitate 8, however, presented a greater challenge. Model reactions on unlabeled 7 allowed us to dismiss the classical Wolf-Kishner reduction, as modified by Sarel and Yanuka (15) for application to the deoxygenation of methyl ketocholanoates. In our case hydrazine reacted too readily with the ester, affording labile hydrazides and unidentifiable subsequent decomposition products. Hydrogenolysis with platinum black, as described by Rylander (16) for the conversion of ethyl acetoacetate into ethyl butyrate, gave the 3-hydroxy analog of 8 contaminated with ethyl hexadec-2-enoate. On the other hand, with p-toluenesulfonylhydrazine as the reagent of choice, the keto ester 7 reacted smoothly as described by Hutchins *et al.* (11). Hydrazone formation and *in situ* reduction with sodium cyanoborohydride produced the fatty acid ester 8 in 75% crude yield as part of a "one-pot" sequence.

Saponification with aqueous sodium hydroxide and precipitation with hydrochloric acid gave labeled palmitic acid 9 in 80% yield after low temperature recrystallization from acetone. Its chromatographic behaviour by TLC and GLC was identical to that of authentic unlabeled material; proton and carbon NMR spectra showed all the expected resonances.

The scheme presented here should be generally applicable to the synthesis of fatty acids with at least four labeled carbons derived from [$^{13}C_4$]acetoacetate. No special skills are required for the route to bear yield in the hands of biochemists investigating lipid metabolism. But one caveat should be noted. The 3-oxo-fatty acid ester 7 is sensitive to hydrolysis and subsequent decarboxylation (17). In one trial, for example, we obtained product contaminated with 2-pentadecanone 10 which on subsequent reductive deoxygenation afforded pentadecane 11. Care should be taken, therefore, in the work up and distillation of 7 to maintain neutral pH conditions.

EXPERIMENTAL

General: Gas chromatographic analyses were performed on a Carle AGC-111 fitted with a flame ionization detector. NMR spectra were recorded using an IBM NR80F spectrometer and IR spectra on a Perkin Elmer 299 spectrometer. ^{13}CO at 90% atom percent enrichment was purchased from Mound Laboratories and used to prepare [^{13}C]methanol and [$^{13}C_2$]acetic acid as described in Ott's handbook (18).

Ethyl [1,2,3,4-$^{13}C_4$]acetoacetate (2): Prepared according to DeGraw et al. (6) in 65% yield after distillation at 50-52°C/6 mm Hg. ^{1}H-NMR ($CDCl_3$) δ: 1.3 (t, J=7 Hz, $\underline{CH_3}CH_2O$), 2.3 (ddd, J_{CH}=130 Hz, J_{CCH}=7 Hz, J_{CCCH}=1 Hz, $^{13}\underline{CH_3}CO$), 3.5 (dt, J_{CH}=132 Hz, J_{CCH}=7 Hz, 2-$^{13}\underline{CH_2}$) and 4.2 ppm (dq, J=7 Hz, J_{COCH}=3 Hz, $O\underline{CH_2}$). (Integration showed the presence of 90% ^{13}C on each labeled carbon).

[1-^{13}C]dodecanoic acid (6): To a one liter autoclave provided with vigorous rocking, heating, cooling and pressurizing means we added a charge of 250 g of methylisobutyl ketone, water (12.5g, 0.694 mol) and undecene (38.5g, 0.25 mol). The liquid mixture was degassed, and the void space replaced with argon. We then added stannous chloride dihydrate (6.1g, 0.025 mol) with stirring followed by bis(triphenylphosphine) palladium (II) chloride (1.75g, 0.0025 mol). After the dissolution of all solids, the reactor was evacuated once again,

pressurized to 1700 psi with ^{13}CO, and heated to a constant temperature of 70°C for 4 hours. The reaction was terminated by cooling the reactor and venting off the reaction gases in such as way as to trap the unreacted ^{13}CO in an evacuated reservoir chilled by liquid nitrogen. The reddish brown solution remaining in the autoclave was transferred to a round bottomed flask with the aid of diethyl ether and concentrated to a syrup under reduced pressure. This was slurried with water, acidified to pH 2 with concentrated HCl and extracted exhaustively with benzene. After drying over anhydrous sodium sulfate, the benzene was removed under reduced pressure to yield crude product. One low-temperature recrystallization from acetone at -63°C gave 36.7g (73%) 4 as a light tan solid, mp 42-46°C, IR (KBr): 1730 (-^{13}C=O acid); NMR ($CDCl_3$) δ: 0.9 (t, J=5 Hz, CH_3), 1.2-1.7 (M, CH_2) and 2.3 ppm (f, J=6 Hz, J_{CCH}=6 Hz; $\underline{CH}_2{}^{13}CO_2H$); CMR(CD Cl_3)δ: 176.3 ppm (s, $\underline{C}OOH$).

1-[1-^{13}C]bromododecane (6): We reduced 4 (20.1g, 100 mmol) with diborane by the literature procedure (13) to obtain crude dodecanol 5, which was converted without further purification into 6 by treatment with 48% HBr as described by Sparrow *et al.* (5). The yield was 19.3g (77%) after column chromatographic purification, on silica gel with hexane as eluant, and short path vacuum distillation, bp 131-136°C/6mm Hg. NMR ($CDCl_3$) δ: 0.9 (t, J=4 Hz, $\underline{CH}_3$), 1.1-2.2 (chain $\underline{CH}_2$) and 3.4 ppm (dt, J=6 Hz, J_{CH}=154 Hz, $^{13}\underline{CH}_2$ Br, 10% uncoupled signal).

Ethyl 3-oxo-[1,2,3,4,5-$^{13}C_5$]hexadecanoate (7): Ethyl [1,2,3,4-$^{13}C_4$]acetoacetate (4.8g, 0.036 mole) was added dropwise to a rapidly stirred slurry of sodium hydride (0.92g, 0.036 mole) in tetrahydrofuran (200 ml) under a nitrogen atmosphere at 0°C. Upon completion of hydrogen evolution after 30 minutes we next began to add slowly a solution of n-butyllithium in hexane (2.2 M, 17.5 ml). Dianion formation was marked by the appearance of a bright orange color, and 30 minutes thereafter we added the dodecylbromide (6) (8.9g, 0.036 mole) in

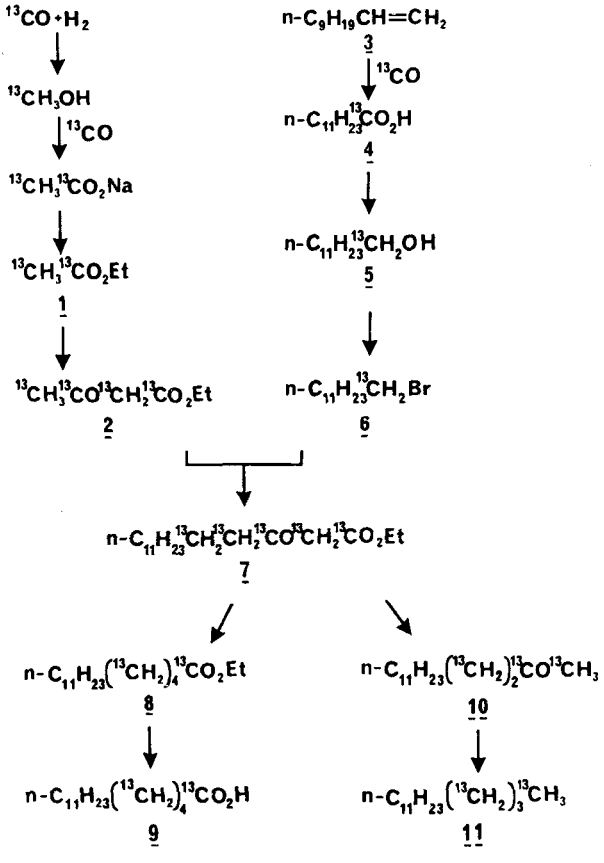
20 ml of tetrahydrofuran. This reactant must be introduced dropwise in order to maintain a reaction temperature at 0 ± 4°C. One hour later, we quenched the reaction mixture carefully with a chilled solution of concentrated HCl (10 ml) in 50 ml of water until pH neutrality had been reached. Repeated ether extractions afforded an organic layer which was dried over sodium sulfate and evaporated to a residue. It was fractionally distilled under reduced pressure for recovery of unreacted acetoacetate 2 and alkyl bromide 6 in addition to 6.9g of product (bp 140-150°C/0.1 mm Hg, 60% conversion, 73% yield based on consumed reactants). A replicate synthesis afforded a second lot of product (6.5g), and the two were combined. NMR ($CDCl_3$) δ: 0.9 (t, J=5 Hz, ω-$\underline{CH_3}$), 1.1-1.8 (m, chain $\underline{CH_2}$), 1.3 (t, J=7 Hz, OCH_2 $\underline{CH_3}$), 1.5 (dm, J_{CH}=130 Hz, 5-$^{13}\underline{CH_2}$), 2.3 (dm, J_{CH}=130 Hz, 4-$^{13}\underline{CH_2}$), 3.5 (dt, J_{CH}=130 Hz, J_{CCH}=6 Hz, 2-$^{13}\underline{CH_2}$) and 4.2 ppm (dq, J=7 Hz, J_{COCH}=3 Hz, $O\underline{CH_2}$).

Ethyl [1,2,3,4,5-$^{13}C_5$]Hexadecanoate (8): The literature procedure (11) was used without modification to give 6.0g (75%) of ester 8 after distillation (bp 129-135/0.1 mm Hg) of the combined crude products obtained from two separate reductive deoxygenations on 4.2g lots (0.013 mole) of 7. NMR ($CDCl_3$) δ: 0.9 (t, J=5 Hz, ω-$\underline{CH_3}$), 1.1-1.6 (m, chain $\underline{CH_2}$ and $OCH_2\underline{CH_3}$), 1.1-1.6 (dm, J_{CH} 130 Hz, 3,4,5-$\underline{CH_2}$'s), 2.1 (dm, J_{CH}=130 Hz, 2-$\underline{CH_2}$) and 4.1 ppm (dq, J=7 Hz, J_{COCH}=3 Hz, $O\underline{CH_2}$).

[1,2,3,4,5-$^{13}C_5$]hexadecanoic acid (9): The ester 8 was saponified in 500 ml of 1% NaOH for 4 hours, and 5g of 9 (90%) was recovered from the chilled reaction mixture after acidification with HCl and filtration. Repeated recrystallizations from acetone afforded a highly purified analytical sample (3g) indistinguishable from authentic palmitic acid (Sigma) by thin layer chromatography (silica gel, 5% acetone in chloroform, Rf = 0.34) or by gas liquid chromatography (GP 5% DEGS-PS on 100/200 Supelcoport, 6 ft packed

column, N_2 carrier, 180°C, Rt = 7 min). NMR ($CDCl_3$) δ : 0.9 (t, J=5 Hz, ω-CH_3), 1.1-1.6 (m, chain $\underline{CH}_2$), 1.1-1.6 (dm, J_{CH} = 130 Hz, 3,4,5-$^{13}\underline{CH}_2$), 2.2 (dm, J_{CH} 130 Hz, $^{13}\underline{CH}_2{}^{13}CO$) and 9.9 ppm ($\underline{OH}$). CMR ($CDCl_3$) δ : 22.3-29.8 (8 peaks, C_3 to C_5 plus CH_2 spike), 34.3 (q, J_{CC}=54.9 and 32.2 Hz, C_2) and 179.7 ppm (d, J_{CC}=54.8 Hz, CO_2H).

Scheme 1



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