SYNTHESES WITH STABLE ISOTOPES: OLEIC-1-^{13}C ACID AND TRIOLEIN-1',1'',1'''-$^{13}C_3$

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SUMMARY

A synthesis of oleic-1-^{13}C acid and its conversion to triolein-1',1'',1'''-$^{13}C_3$ are described. 9-Octadecynenitrile-1-^{13}C was prepared from 1-bromo-8-heptadecyne and sodium cyanide-^{13}C. Hydrolysis afforded stearolic-1-^{13}C acid, which was reduced to oleic-1-^{13}C acid by the action of disiamylborane on methyl stearolate-1-^{13}C. Hydrogenation of stearolic acid in the presence of Lindlar's catalyst was also investigated. Condensation of glycerol and oleic-1-^{13}C acid to give triolein-1',1'',1'''-$^{13}C_3$ was effected with dicyclohexylcarbodiimide and 4-dimethylaminopyridine. The overall yield of triolein-$^{13}C_3$ from sodium cyanide-^{13}C was 40%.

Key Words: triolein-1',1'',1'''-$^{13}C_3$, oleic-1-^{13}C acid, carbon-13

INTRODUCTION

There have been several reports of the use of triglycerides labeled with carbon isotopes to detect fat malabsorption conditions by breath tests. A breath test using carbon-13 labeled trioctanoin has been described by Watkins, et al.[1] A comparative study of triolein, tripalmitin, and trioctanoin using carbon-14 labeled triglycerides has been reported by Newcomer, et al.[2] This study suggested that the unsaturated triglyceride is superior for detecting fat malabsorption. It appears that triolein labeled with the stable carbon isotope is the most desirable substrate for clinical studies. We have prepared carbon-13 labeled triolein for additional studies and wish to report its synthesis.

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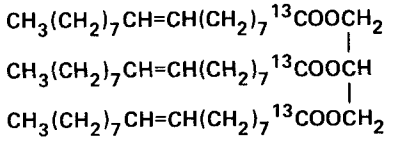
RESULTS AND DISCUSSION

The carboxyl groups of the fatty acid residues in triolein are the most convenient positions for labeling of the triglyceride. The synthesis of triolein-1',1'',1'''-$^{13}C_3$ (1) requires either oleic-1-^{13}C acid (2) or an ester such as methyl oleate-1-^{13}C (3) as an immediate precursor. One



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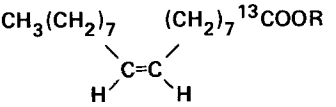
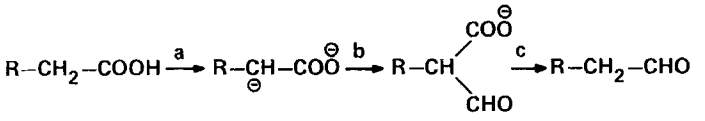


2 R=H

3 R=CH_3

approach to the labeling pattern in 2 or 3 is a carbonyl-replacement sequence (i.e., a synthesis whose overall transformation is the replacement of the carbonyl carbon of oleic acid with isotopic carbon). Oleic-1-^{14}C acid has been prepared by such sequences [3,4]; however, the protection and deprotection of the double bond in these synthesis does not proceed with stereochemical integrity. Initially, we investigated the carbonyl-replacemet sequence shown in scheme I which does not require

SCHEME I



4 5 6 7

R = $CH_3(CH_2)_7CH{=}CH(CH_2)_6^-$

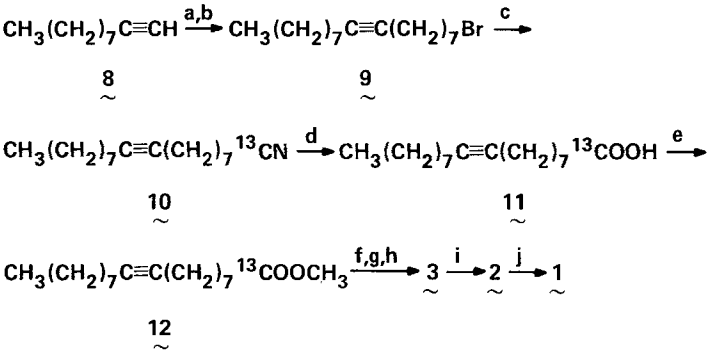
(a) LDA, THF; (b) HCOOEt; (c) $-CO_2$

protection of the double bond. Oleic acid (4) was converted to its dianion 5[5] and condensed with ethyl formate[6] to give the α-formyl derivative 6. Upon decarboxylation 6 afforded olealdehyde (7). In our hands, this

sequence gave low yields (ca. 30%) of product that was contaminated with oleic acid and other unidentified products. Similar condensations of 5 with ethyl chloroformate and diethyl carbonate[7] were also found to be unsuitable. As a result, this approach was abandoned.

Our second approach to 1 is shown in Scheme II. The isotopic label in

SCHEME II



(a) BuLi, THF; (b) $Br(CH_2)_7Br$; (c) $Na^{13}CN$, DMSO; (d) KOH, EtOH-H_2O
(e) $(CH_3)_2C(OCH_3)_2$, HCl, MeOH; (f) disiamylborane, THF; (g) HOAc;
(h) H_2O_2; (i) NaOH, EtOH-H_2O; (j) glycerol, DCC, DMAP, Et_2O.

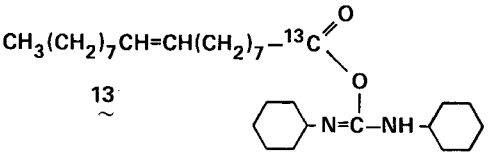
oleic-1-^{13}C acid was introduced via a nitrile synthesis. The requisite bromide 9 was synthesized by the reaction of the lithium salt of 1-decyne (8) with 1,7-dibromoheptane. In order to suppress bisalkylation 1,7-dibromoheptane was used in excess, unreacted dibromide being easily recovered by distillation. Attempts to carbonate the Grignard reagent of 9 were unsatisfactory because of low yields. 9-Octadecynenitrile-1-^{13}C (10) was obtained in 80% yield from the reaction of the bromide 9 with sodium cyanide-^{13}C in dimethyl sulfoxide.[8] Basic hydrolysis of 10 gave stearolic-1-^{13}C acid (11).

Initially, we attempted to reduce the triple bond of stearolic acid to the cis double bond of oleic acid by hydrogenation using Lindlar's catalyst

Pd/$CaCO_3$) moderated by synthetic quinoline.[9] Several problems were ncountered. Despite numerous preparations of the catalyst, we were unable b obtain catalyst with the reported activity.[10] Furthermore, as could b shown by gas chromatography or thin layer chromatography after esterfication of the reaction mixture, the Lindlar reduction of stearolic acid ave oleic acid contaminated with stearolic acid, stearic acid, and elaidic cid at the level of several percent. It could be shown that the trans somer (elaidic acid) arises by isomerization of the cis isomer (oleic cid) by the catalyst and not by direct reduction of stearolic acid. This esult has been observed in other semihydrogenation reactions.[11] We were nable to find a convenient method for purifying oleic acid obtained from he Lindlar reduction. Attempts to purify methyl oleate by low temperature rystallization were unsuccessful. Chromatography on a silica gel column ith 95:5 (v/v) hexane-ether eliminated methyl stearolate and methyl tearate but did not resolve methyl oleate and methyl elaidate. Chromatography[12] on an Amberlyst XN-1010 resin column (Ag^+, 60-80 mesh, dution with MeOH) separated methyl elaidate, but did not resolve methyl leate and methyl stearolate.

Successful reduction of the triple bond in 11 was accomplished by a ydroboration procedure. Stearolic-1-^{13}C acid was first converted to the ethyl ester 12 with 2,2-dimethoxypropane.[13] Reduction of the triple ond was carried out using disiamylborane in tetrahydrofuran.[14] Overeduction, incomplete reaction, or trans stereochemistry were not observed ith the organoborane reagent. After reduction, protonolysis, and oxiative work up, methyl oleate-1-^{13}C was saponified to give the acid 2 in uantitative yield from 11. Conversion of 2 to triolein was accomplished y condensing 2 and glycerol using dicyclohexylcarbodiimide (DCC) and 0 mol% of 4-dimethylaminopyridine (DMAP) in ether.[15] By using a lycerol:2:DCC ratio of 1:4:8, the reaction proceeds to completion without iolein products. Condensations in pyridine are less satisfactory. llowing precipitations to remove dicyclohexylurea (DCU), the reaction

mixture was passed through a silica gel column to remove DCC and DCU. Final purification was accomplished by preparative-scale liquid chromatography. The excess 2 used in the condensation reaction was recovered as the O-acylisourea 13. The structure of 13 was assigned on the basis of spectral evidence and elemental analysis.



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EXPERIMENTAL

Materials and Methods--1-Decyne was obtained from Chemical Sample Co. 1,7-Dibromoheptane, 2,2-dimethoxypropane, 2-methyl-2-butene, and borane-THF were obtained from Aldrich Chemical Co. The following authentic compounds were obtained for spectral and chromatographic comparisons: oleic acid and triolein (Tridom Chemical Co); elaidic acid (Aldrich Chemical Co); stearolic acid (Thiokol/Ventron Division, Alfa Products); 1-monoolein, 2-monoolein, 1,2-diolein, and 1,3-diolein (P-L Biochemicals). Sodium cyanide-^{13}C was produced at this laboratory.[16] Thin layer chromatography was conducted with 5 x 20-cm silica gel 60 glass plates using hexane-ethyl acetate (77:23, v/v) as a developing solvent. Unsaturated compounds were detected with I_2 vapor. Compounds analyzed and their approximate R_f's were 1 (0.64), 13 (0.39), 1,3-diolein (0.28), 1,2-diolein (0.22), and 2 (0.10). Gas chromatography was conducted with a Hewlett-Packard Model 5710A gas chromatograph using either a 10-m x 0.25-mm ID SP-2100 capillary column (column pressure 20 psig) and flame ionization detector or a 6'x 1/8" 3% OV-17 packed column (He flow 24 mL/min) and thermal conductivity detector. Other conditions were: injection port temperature of 250°C; detector temperature 300°C; and temperature programming from 80-220°C at 8°C/min. Compounds analyzed on the OV-17 column and their approximate retention times

were: 8 (1.6 min); $Br(CH_2)_7Br$ (8.1 min); 9 (16.7 min); and 10 (19 min). Compounds analyzed on the SP-2100 column and their approximate retention times were: 3 (13.4 min); 12 (13.8 min); 2 (14.1 min); and 11 (14.5 min). Melting points were measured with a Fischer-Johns apparatus and are uncorrected. ^{13}C NMR spectra of $CDCl_3$ solutions were recorded at 25°C with a Varian Model CFT-20 spectrometer. Peaks were referenced to solvent $CDCl_3$ at 76.9 ppm and are reported relative to TMS. Infrared spectra were recorded with a Perkin-Elmer Model 283 spectrophotometer. Preparative liquid chromatography was conducted with a Waters Associates Prep LC/System Model 500A using a single PrepPAK-500 Silica cartridge.

1-Bromo-8-heptadecyne (9)--A solution of 1-decyne (40.6 g, 0.29 mol) in anhydrous THF (500 mL) was introduced into a 3-neck flask that had been flushed with N_2 and was equipped with a mechanical stirrer, addition funnel, and condenser. After cooling the decyne solution in an ice bath, butyllithium in hexane (199 mL, 1.46 M, 0.29 mol) was added dropwise over 30 min. The ice bath was removed and a solution of 1,7-dibromoheptane (155 g, 0.58 mol) in anhydrous THF (400 mL) was added dropwise over 15 min. The reaction mixture was then heated at reflux for 24 h. Upon cooling, the reaction mixture was poured into water (500 mL). The aqueous layer was removed and extracted with CH_2Cl_2 (3 x 100 mL). The organic phase from the reaction mixture was reduced in volume to ca. 300 mL and extracted with water (3 x 100 mL). The combined aqueous phases were back-extracted once with CH_2Cl_2 (100 mL). The organic layers were combined and dried over anhydrous $MgSO_4$. The solvent was removed by rotary evaporation, and the resulting liquid was distilled at reduced pressure through a 15-cm unpacked column to give 86.3 g of recovered 1,7-dibromoheptane (bp 84-86°C, 1 torr) and 72.3 g (79%) of 9 as a clear liquid (bp 156-158°C, 1 torr): ^{13}C NMR ($CDCl_3$) δ 80.1, 79.7, 33.6, 32.6, 31.7, 29.0, 28.8, 28.7, 28.4, 28.1, 27.9, 22.5, 18.5, 13.9; IR (neat) 2930, 2850, 1465, 1435, 720 cm^{-1}.

9-Octadecynenitrile-1-^{13}C (10)--A 3-neck flask equipped with a mechanical stirrer, addition funnel, condenser with $CaCl_2$ drying tube, and

thermometer was charged with sodium cyanide-^{13}C (7.55 g, 94% NaCN, 93 mol% ^{13}C, 0.14 mol) and DMSO (100 mL). The mixture was heated to 60°C, and the bromide 9 (44.6 g, 0.14 mol) was added dropwise over 30 min such that the temperature did not exceed 70°C. The addition funnel was rinsed with DMSO (50 mL), and the reaction mixture was maintained at 70°C for 7 h. The stirred mixture was cooled in an ice bath and diluted with water (250 mL). The mixture was extracted with CH_2Cl_2 (5 x 100 mL), and the combined extracts were dried over anhydrous $MgSO_4$. The solvent was removed by rotary evaporation, and the residue was distilled at reduced pressure to give 29.9 g (80%) of 10 as a clear liquid (bp 157-159°, 1 torr): ^{13}C NMR ($CDCl_3$) δ 119.5 ($^{13}C{\equiv}N$), 80.2 and 79.6 (C≡C); IR (neat) 2940, 2860, 2190 ($^{13}C{\equiv}N$), 1465, 1435, 725 cm^{-1}.

Stearolic-1-^{13}C Acid (11)--A solution of aqueous ethanol (70% EtOH, v/v, 184 mL), KOH (24.3 g), and the nitrile 10 (48.4 g, 0.18 mol) was heated at reflux for 24 h. After cooling, most of the EtOH was removed by rotary evaporation. The resulting potassium stearolate-1-^{13}C solution was acidified by dropwise addition, over a period of 1 h, to 5% HCl (1 L), which was cooled in an ice bath and stirred during the addition.[17] The resulting solid was filtered, washed with water, and dried under reduced pressure to give 50.6 g of crude 11 as a colorless solid, mp 43.5-45°C. Azeotropic removal of water from the crude product was effected by dissolution in ethyl acetate and boiling at atmospheric pressure. Evaporation gave a solid that was crystallized from MeOH to give, in two crops, 44.3 g (85%) of 11 as white needles, mp 45.5-46°C (reported[18] 47-48°C): ^{13}C NMR ($CDCl_3$) δ 180.1 ($^{13}COOH$), 80.2 and 80.0 (C≡C), 33.9 ($\underline{C}H_2{}^{13}COOH$, $^1J_{CC}$ = 55.4 Hz); IR (KBr) 2950, 2930, 2850, 1650 ($^{13}COOH$), 1465, 1270, 915, 720 cm^{-1}.

Methyl Stearolate-1-^{13}C (12)--A solution of the acid 11 (64.2 g, 0.23 mol), MeOH (500 mL), concentrated HCl (12.8 mL), and 2,2-dimethoxypropane (327 mL) was prepared and allowed to stand at room temperature for 2 h. Solvent was removed by rotary evaporation. Residual HCl was removed

by repeated addition and evaporation of MeOH (8 x 50 mL). The ester 12 was obtained as a colorless oil (50.2 g, 74.5%, ca. 99% pure by gc) and was used without purification[19]: ^{13}C NMR ($CDCl_3$) δ 174.0 ($^{13}\underline{C}OOCH_3$), 80.1 and 79.9 (C≡C), 51.2 ($\underline{C}H_3O^{13}C$, $^2J_{CC}$ = 2.8 Hz); IR (neat) 2920, 2850, 1740 ($^{12}COOCH_3$), 1695 ($^{13}COOCH_3$), 1460, 1430, 1150, 720 cm^{-1}.

Oleic-1-^{13}C Acid (2)--A solution of the ester 12 (50.1 g, 0.17 mol) in anhydrous THF (50 mL) was introduced into a 3-neck flask equipped with a stirring bar, condenser, addition funnel with septum, and septum on one neck and maintained under a N_2 atmosphere. The reaction flask was then cooled to -10°C. A freshly prepared[20] solution of disiamylborane in anhydrous THF (415 mL, 0.4 M, 0.17 mol) was introduced into the addition funnel. The disiamylborane solution was added dropwise over 2 h to the solution of 12 with the temperature of the reaction mixture maintained below -5°C. After the addition was complete, the cooling bath was removed, and the reaction mixture was stirred at room temperature for 2 h. At this time, analysis[21] showed that unreacted 12 was present. Additional disiamylborane solution (42 mL, 0.4 M, 17 mmol) was added, and stirring at room temperature was continued for 1 h. The reaction mixture was cooled in an ice bath, acetic acid (100 mL) was added, and the mixture was stirred for 8 h at room temperature. The volume of the mixture was reduced to ca. 200 mL and then the mixture was diluted with water (100 mL) and THF (300 mL). While stirring, this mixture was then titrated to pH 7 with 2 N NaOH and then made basic by the addition of 2.0 N NaOH (93.5 mL, 0.187 mol). The resulting mixture was cooled in an ice bath, and 30% H_2O_2 (51 g, 0.23 mol) was added dropwise over 15 min to oxidize disiamylborinate. This mixture separated into two layers. The organic layer was extracted with water until the extracts were no longer basic. The combined aqueous extracts (ca. 1.8 L) were back-extracted with hexane (6 x 100 mL), and the combined organic phases were dried over anhydrous $MgSO_4$. Removal of the solvent by rotary evaporation afforded the ester 3 (53.8 g) as a colorless liquid. Saponification of 3 was accomplished with NaOH (15 g, 0.37 mol) in aqueous

EtOH (70% EtOH, v/v, 190 mL) at reflux for 3 h. The hydrolysis reaction mixture was concentrated to ca. 100 mL and was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were dried over anhydrous $MgSO_4$. Evaporation of the solvent gave the acid 2 as a pale yellow oil (48.1 g, 100%, ca. 99% pure by gc), which was used without purification: ^{13}C NMR ($CDCl_3$) δ 180.0 ($^{13}COOH$), 130.4 and 130.1 (cis CH=CH), 34.4 ($\underline{CH}_2{}^{13}COOH$, $^1J_{CC}$ = 54.9 Hz); IR (neat) 3000, 2920, 2850, 1670 ($^{13}COOH$), 1465, 1270, 1210, 720 cm^{-1}.

Triolein-1,1'',1'''-^{13}C (1)--A solution of anhydrous glycerol (3.89 g, 42 mmol), 2 (48.0 g, 0.17 mol), and DMAP (2.1 g, 17 mmol) in anhydrous Et_2O (100 mL) was prepared in a round-bottom flask contained in a glove box flushed with N_2. Cautiously, DCC (69.7 g, 0.34 mol) and additional Et_2O (240 mL) were added. The flask was stoppered, and the mixture was stirred at room temperature for 70 h. The reaction mixture was filtered to remove precipitated DCU, and the solvent was removed from the filtrate. The residue was taken up in hexane (200mL) and cooled to crystallize more DCU, which was removed by filtration. The solvent was removed from the filtrate to give 90.1 g of a mixture of 1, 13, DCC, DCU, and DMAP. A portion of this mixture (50.8 g) was dissolved in hexane (125 mL) and applied to a 7 x 68-cm column of silica gel 60 (0.063-0.2 mm particle size). Elution with hexane-ethyl acetate (77:23, v/v) was conducted at a flow rate of ca. 3 mL/min. The DCC and DCU remained at the top of the column and were visually apparent. The initial 1.76 L was collected and discarded. Triolein and 13 were collected in the next 2.56 L. A final fraction of 3.7 L was found to contain 13 and some oleic-1-^{13}C acid (2). The remaining 1-13-DCC-DCU-DMAP mixture was treated in a similar fashion. From the total mixture, 50.7 g of a 1-13 mixture and 4.7 g of a 13-2 mixture were obtained. The triolein-isourea mixture was separated by preparative liquid chromatography. For a single run, 10 g of the 1-13 mixture was dissolved in 10 mL of hexane-ethyl acetate (9:1, v/v), injected onto the column, and eluted with the same hexane-ethyl acetate solvent. From the total mixture,

28.8 g of 1 and 18.7 g of 13 were obtained (80% yield of 1 based on recovered 13). Triolein-1',1'',1'''-$^{13}C_3$ was a pale yellow oil: ^{13}C NMR ($CDCl_3$) δ 173.1 ($^{13}\underline{C}OOCH_2$), 172.6 ($^{13}\underline{C}OOCH$), 129.9 and 129.6 (CH=CH), 68.7 ($\underline{C}HOO^{13}C$), 61.9 ($\underline{C}H_2OO^{13}C$), 34.0 ($\underline{C}H_2{}^{13}COOCH$, $^1J_{CC}$ = 57.4 Hz), 33.8 ($\underline{C}H_2{}^{13}COOCH_2$, $^1J_{CC}$ = 57.6 Hz); IR (neat) 3000, 2920, 2850, 1705 (^{13}COOR), 1465, 135, 720 cm^{-1} (no traces of DCC or DCU in IR); single spot on tlc. The O-acylisourea 13 was also a pale yellow oil: ^{13}C NMR δ 173.2 (^{13}COOR), 153.8 ($N\underline{C}(=N)OO^{13}C$, $^2J_{CC}$ = 4 Hz), 129.7 and 129.4 (CH=CH), 55.4 (C-N), 49.5 (C-N), 35.5 ($\underline{C}H_2{}^{13}COOR$, $^1J_{CC}$ = 50.9 Hz); IR 3290 (N-H), 2920, 2850, 1710 (^{13}COOR), 1605 (C=N), 1530 cm^{-1}; Anal. Calcd for $C_{31}H_{56}N_2O_2$ containing one ^{13}C at 93 mol % ^{13}C: C 76.22, H 11.53, N 5.72. Found C 76.47, H 11.57, N. 5.66.

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17. Addition of acid to the basic solution or rapid addition of the salt to acid usually gave an oil instead of a solid product.

18. Heilbron I., Ed., - Dictionary of Organic Compounds, Vol. 4, Oxford University Press, New York, 1953, p. 372.

19. The yield given here is probably atypical. In other reactions, yields greater than 90% were realized.

20. The disiamylborane solution was prepared from BH_3-THF solution and 2-methyl-2-butene in THF. Brown H. C. - Organic Synthesis via Boranes, Wiley, New York, 1975, p. 29.

21. For analysis, 20 µL of the reaction mixture was added to 200 µL of HOAc and heated at 50°C for 15 min. Analysis by capillary column gc showed 12, 2, and an intermediate complex (retention time = 15.6 min).