SYNTHESIS OF CARBON-14-LABELED SODIUM PALMOXIRATE AND ITS COENZYME A ESTER

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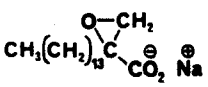
SUMMARY

Synthetic procedures for the preparation of carbon-14-labeled sodium palmoxirate (TDGA), labeled either in the carboxyl position or in the tetradecyl hydrocarbon chain, are described. In addition, the synthesis of the coenzyme A ester of TDGA-^{14}C with a specific activity of 51 mCi/mmol is reported. The coenzyme A ester was prepared by formation of the acyl chloride with oxalyl chloride followed by reaction with coenzyme A (CoA) in a borate-buffered tetrahydrofuran solution. Purification methods and analytical and stability data are reported for the compounds.

Key Words: Sodium Palmoxirate, Sodium 2-Tetradecylglycidate, Coenzyme A Ester, Fatty Acid, Carbon-14, Synthesis

INTRODUCTION

Sodium palmoxirate (1, sodium 2-tetradecylglycidate, TDGA, sodium 2-tetradecyloxiranecarboxylate) and its methyl ester are potent, orally active inhibitors of long-chain fatty acid oxidation. They have been shown to be effective hypoglycemic and antiketogenic compounds in both animals (1) and man (2), and are currently of interest in studying the relationship of fatty acid oxidation to various other metabolic processes such as gluconeogenesis (1-7).



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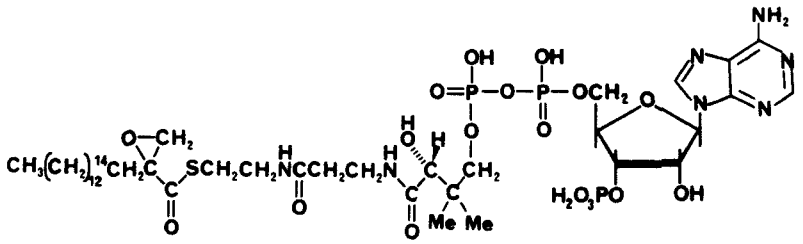

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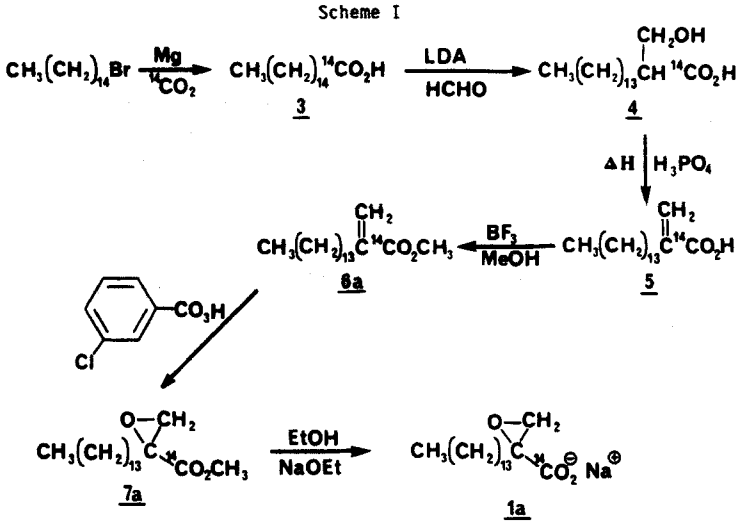
To gain further insight into the mechanism of action and metabolic fate of TDGA, carbon-14-labeled samples of 1 and the CoA ester of TDGA (2) were prepared. TDGA was tagged either in the carboxyl group or in the tetradecyl sidechain using two different synthetic schemes. The carboxyl-labeled material was synthesized to determine if the carboxyl group remained intact or underwent oxidative cleavage during in vivo experiments. The second tagged sample, labeled in the tetradecyl hydrocarbon chain, was used for more extensive metabolic studies in animals. Some of this material was converted to the TDGA-^{14}C-CoA thioester (2) for in vitro mechanism studies (3).

RESULTS AND DISCUSSION

The synthesis of TDGA labeled in the carboxyl position (1a) is outlined in Scheme I. Hexadecanoic-1-^{14}C acid (palmitic acid -1-^{14}C, 3) was prepared from 1-bromopentadecane via a Grignard reaction with $^{14}CO_2$ which was generated on a vacuum system by the addition of concentrated sulfuric acid to dry $Ba^{14}CO_3$. Conversion to 2-tetradecylacrylic acid 5, using the procedure of Pfeffer et al. (8), was carried out by treatment of 3 with lithium diisopropylamide in tetrahydrofuran (THF) followed by reaction of the lithium carboxylate dianion with formaldehyde to give α-alkylhydracrylic acid 4. Dehydration of 4 was completed by heating at 180°C in the presence of a catalytic amount of phosphoric acid followed by distillation at 270°C in vacuo. The distilled product was purified by column chromatography and esterified with boron trifluoride in methanol to give acrylic ester 6a. Compound 6a was oxidized with m-chloroperoxybenzoic acid to oxirane 7a according to a previously described procedure (1). Hydrolysis of 7a with sodium ethoxide in aqueous ethanol

Scheme I

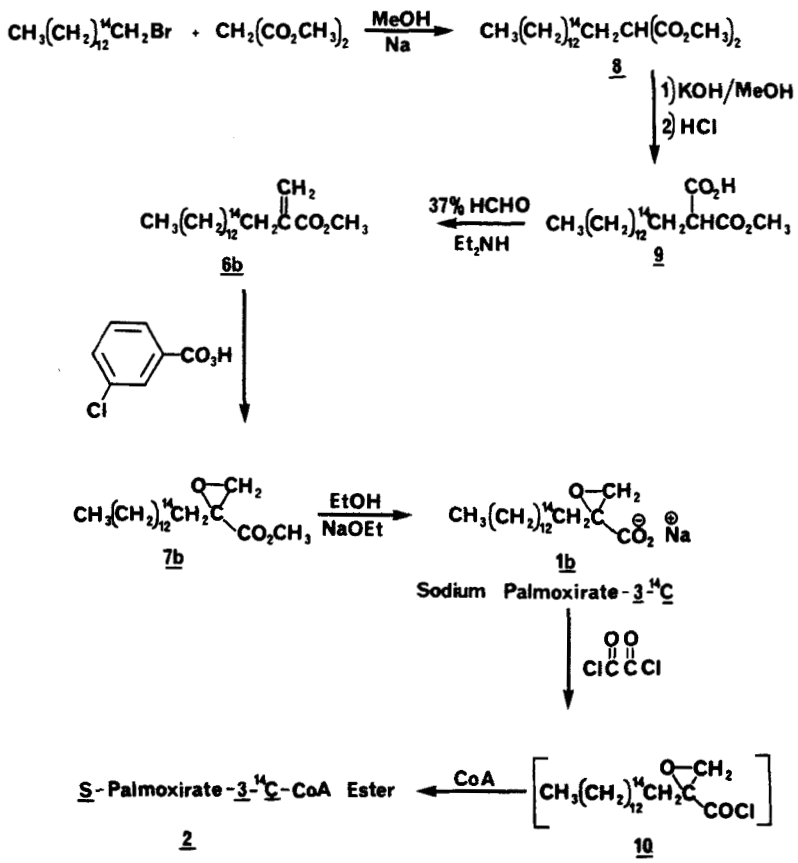


provided TDGA (1a) labeled with carbon-14 in the carboxyl position. The isolated product had a specific activity of 2.08 mCi/mmol and a radiochemical purity greater than 98%.

The synthetic route used to prepare TDGA (1b) labeled in the tetradecyl hydrocarbon chain and the corresponding CoA ester (2) is shown in Scheme II. Labeled tetradecylacrylic acid 6b was prepared from 1-bromotetradecane-1-^{14}C in a manner similar to the procedure employed by Gisser and Mertway (9), and Ho et al (1). The sequence involved condensation of carbon-14-labeled 1-bromotetradecane with dimethyl malonate to give diester 8. Alkaline hydrolysis to the half acid-ester (9) followed by reaction with aqueous formaldehyde and diethylamine provided 6b in excellent yield. Conversion of 6b to the desired labeled TDGA (1b) was completed by epoxidation with m-chloroperoxybenzoic acid and hydrolysis with sodium ethoxide as mentioned above. The final product had a specific activity of 51 mCi/mmol and chemical and radiochemical purities in excess of 98%.

A portion of the labeled TDGA-3-^{14}C (1b) was employed in the preparation of the CoA ester (2). This was completed by treatment of 1b with oxalyl chloride to give acyl chloride 10 followed by reaction with coenzyme A.

Scheme II



Numerous methods have been described for the synthesis of fatty acid coenzyme A esters (10-16), and the preparation of carbon-14-labeled CoA esters has been previously reported (11,17). However, because of the sensitive epoxide functionality in TDGA, it was necessary to choose a synthesis involving mild conditions. The procedure selected for our purposes involved the preparation of acid chloride 10 with oxalyl chloride followed by reaction with CoA. The conditions were essentially those described by Seubert (10), but modified to reduce the 70:1 ratio of labeled acyl chloride to coenzyme A. The final ratio that was employed was 15:1, acyl chloride to CoA. The reaction facilely pro-

vided the desired product at this reactant ratio. Attempts to use the procedure of Bishop and Hajra (11), which employed an excess of coenzyme A and inverse addition of the reactants for the efficient preparation of carbon-14-labeled palmitoyl coenzyme A, gave no detectable product with TDGA (1). The procedure, however, was effective with palmitic acid.

Chemical yields of up to 79% based on CoA were obtained using the 15:1 reactant ratio. Early experiments, however, gave yields that varied between 10 and 70%; these results were determined to be due to difficulties in maintaining the pH of the microscale reaction in the range of pH 8-9, and incomplete removal of water from TDGA. Sodium tetraborate (borax) was found to be an efficient buffer which eliminated manual addition of base during the course of reaction. The complete removal of water during formation of the acyl chloride was essential. Drying of glassware and extensive drying of 1b over phosphorous pentoxide *in vacuo* was found to be necessary in order to remove bound water.

EXPERIMENTAL SECTION

Barium carbonate-^{14}C was obtained from New England Nuclear Corp., Boston, MA and 1-bromotetradecane-1-^{14}C was prepared by Amersham Corp., Arlington Heights, IL. Dimethylmalonate, diethylamine, paraformaldehyde, *m*-chloroperoxybenzoic acid (80-85%), *n*-butyllithium (1.6 M in hexane), hexamethylphosphoramide (HMPA), 1-bromopentadecane, boron trifluoride methanol complex, diisopropylamine, oxalyl chloride, sodium tetraborate decahydrate, and mercaptoacetic acid were obtained from Aldrich Chemical Co. (Milwaukee, WI). The 1-bromopentadecane was distilled prior to use on a Nester/Faust spinning band distillation apparatus, the paraformaldehyde was dried over phosphorous pentoxide *in vacuo* at 25°C and the HMPA and diisopropylamine were dried over molecular sieves (type 4A, Union Carbide Corp., Linde Div., New York, NY). Magnesium metal turnings were purchased from Mallinckrodt (St. Louis, MO) and 37% aqueous formaldehyde was obtained from Fisher Scientific (King of Prussia, PA). Coenzyme A was purchased from Sigma (St. Louis, MO) as the sodium salt with a purity of 90-95%.

The proton magnetic resonance spectra were obtained using a Brucker AM 360 instrument. Ultraviolet absorption spectra were measured with a Perkin-Elmer

Lambda 5 spectrophotometer using 1 cm matched cells. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA. Thin-layer chromatography was carried out with silica gel GF plates obtained from Analtech, Inc. (Newark, DE). Radioscans were taken on a Varian-Berthold Series 6000 thin-layer scanner and specific activities were determined on a Searle Isocap/300 liquid scintillation spectrophotometer. Gas chromatography was carried out using a Perkin-Elmer model 3920B instrument with columns obtained from Alltech (Deerfield, IL). A Laboratory Data Control (Riviera Beach, FL) high performance liquid chromatograph equipped with a variable wavelength UV detector was used for analyses.

Hexadecanoic-1-^{14}C Acid (3)

A solution of 1-bromopentadecane (14 mmol) dissolved in 20 mL of diethyl ether was slowly added to a suspension of magnesium metal turnings (18 mmol) in diethyl ether (3 mL). The mixture was heated at reflux temperature for 2.5 h, cooled to -20°C and 11.4 mmol of carbon dioxide-^{14}C, generated by the slow addition of concentrated sulfuric acid to 230 mCi (20.1 mCi/mmol) of barium carbonate-^{14}C, was vacuum transferred into the reaction. The reaction was stirred for 20 minutes and 20 mL of 5% hydrochloric acid was added while maintaining the temperature at -20°C. The mixture was extracted with ether and the organic layer washed with water and dried over anhydrous magnesium sulfate. Filtration and evaporation of the filtrate gave a crude white solid which was chromatographed on silica gel (SilicAR®, CC-7, Mallinckrodt) using a hexane:diethyl ether:glacial acetic acid (70:30:1, v/v) solvent system. The fractions containing product (monitored by tlc in the above system and visualized with iodine vapor) were collected and evaporated to dryness *in vacuo*. The hexadecanoic-1-^{14}C acid (9.0 mmol) was recovered in a 79% yield after drying at 45°C *in vacuo* for three hours. Thin-layer chromatography was carried out on silica gel GF using the above solvent system. Visualization in iodine vapor showed a major spot which chromatographed at the same R_f as reference hexadecanoic acid (R_f 0.55) and a trace impurity at lower R_f (0.36).

2-(Hydroxymethyl)hexadecanoic-1-^{14}C Acid (4)

A solution of *n*-butyl lithium (24.7 mmol) in hexane was added to a cooled solution of diisopropylamine (24.7 mmol) and 30 mL of THF at a rate that main-

tained the temperature below 0°C. After stirring for five minutes, HMPA (22.2 mmol) was added followed by a solution of 10 mmol hexadecanoic-1-^{14}C acid* dissolved in 15 mL of THF. The resulting suspension was heated in a water bath at 45°C to give a clear solution. Formaldehyde vapors, generated in a second flask by heating (29.9 mmol) paraformaldehyde at 180-200°C, were passed into the reaction mixture by a slow stream of nitrogen gas. The reaction was terminated when all of the formaldehyde was transferred. The reaction was neutralized to pH 7 with 1N hydrochloric acid and concentrated to an oily syrup on a rotary evaporator. The residue was extracted into diethyl ether and washed with 10% hydrochloric acid followed by water. The ether layer was separated, dried over magnesium sulfate, and filtered. The filtrate was evaporated to give a white crystalline solid in 80% yield. Tlc:silica gel GF, hexane: diethyl ether:glacial acetic acid (75:25:5, v/v, R_f 0.48).

2-Methylenehexadecanoic-1-^{14}C Acid (5)

An 8.03 mmol sample of 2-(hydroxymethyl)hexadecanoic-1-^{14}C acid (4) and 50 µL of 85% phosphoric acid were placed in a short path microdistillation apparatus equipped with a thermometer and vacuum adapter. The mixture was heated at 180°C/20 torr for 30 min and the resulting dark oil was distilled at 270°C/0.25 torr. The distillate was chromatographed on a silica gel (SilicAR®, CC-7, Mallinckrodt) column using a hexane:diethyl ether:glacial acetic acid (75:25:5, v/v) solvent system. The fractions containing product (determined by tlc) were combined and the solvent evaporated. A white crystalline solid was obtained in 78% yield after drying in vacuo at 45°C for four hours. Thin-layer chromatography in the above system showed the product to be a single component (R_f 0.68).

Methyl 2-Methylenehexadecanoate-1-^{14}C (6a)

A boron trifluoride methanol complex (1.7 mL, 50% boron trifluoride by weight) was added to 6.26 mmol of 2-methylenehexadecanoic-1-^{14}C acid (5) dissolved in 5 mL of absolute methanol. The solution was heated at reflux temperature for five hours and the solvent removed on a rotary evaporator. Satur-

* Labeled 3 (9.0 mmol) from above was diluted with carrier (1.0 mmol) to adjust the reagent quantities to the same amounts as those used during trial runs.

ated sodium bicarbonate solution was added to neutralize the residue and the resulting aqueous mixture was extracted with diethyl ether. The organic layer was separated, washed with water and dried over magnesium sulfate. The solvent was evaporated and the residue chromatographed on a silica gel (Woelm, Universal Scientific Inc., Atlanta, GA) dry column using a hexane:diethyl ether (75:25, v/v) solvent system. The column was segmented and the fractions containing product were extracted with methanol followed by ether and filtered. The filtrates were combined and evaporated to dryness. The product was obtained in a 22% yield after drying *in vacuo* at room temperature for three hours. The material had an R_f of 0.74 on silica gel GF using the above chromatography system.

Methyl 2-Tetradecyloxiranecarboxylate-1-^{14}C (7a)

A mixture of methyl 2-methylenehexadecanoate-1-^{14}C (6a, 1.38 mmol), 30 mL of 1,2-dichloroethane, 15 mg 3-*tert*-butyl-4-hydroxy-5-methylphenylsulfide (Aldrich) and *m*-chloroperoxybenzoic acid (2.8 mmol) was heated at reflux temperature for four hours. The solvent was removed on a rotary evaporator and the residue extracted into ether. The organic layer was washed with a saturated solution of sodium bicarbonate, dried over magnesium sulfate and filtered. The filtrate was concentrated to dryness and the residue chromatographed on a silica gel (Silica AR®, CC-7, Mallinckrodt) column using a hexane:diethyl ether (85:15, v/v) solvent system. The fractions containing product (visualized by using tlc in the above solvent system and spraying with a solution of 7% phosphomolybdic acid in 95% ethanol and heating at 150°C for 15 min) were combined and evaporated to dryness. The resulting white crystalline solid was recrystallized from methanol to give a 70% yield of product after drying at 37°C *in vacuo* for 15 hours (MP 43.5-44.5°C, corrected). Analysis by gas chromatography found the material to have a chemical purity of 99% by area normalization (t_R 9.45 min). Gas chromatography was performed on a 2 m x 2 mm SE-30 on 100/120 mesh Gas Chrom Q glass column heated from 90-280°C at 16°/min, using helium as the carrier gas and a flame ionization detector (FID). Thin-layer chromatography and radioscan showed the material to consist of a major component corresponding to methyl TDGA-1-^{14}C (R_f 0.64) and a higher eluting

0.5% radiochemical impurity on silica gel GF using a mobile phase of hexane:diethyl ether (85:15, v/v). Visualization of the plates was carried out by using the phosphomolybdic acid spray reagent described above.

Sodium Palmoxirate-1-^{14}C (Sodium 2-Tetradecyloxiranecarboxylate-1-^{14}C, 1a)

A mixture of 1.9 mL absolute ethanol and 1.97 mmol sodium metal was stirred at room temperature until the sodium fully dissolved. To this solution was added 0.97 mmol methyl 2-tetradecyloxiranecarboxylate-1-^{14}C (7a) and 0.1 mL water. The reaction mixture was stirred at room temperature for four hours and the resulting precipitate filtered, washed with cold ethanol (1 mL) and slurried in water (2 mL). The resulting suspension was filtered and a white crystalline solid was recovered in 60% yield after drying at 35°C *in vacuo* for 18 hours. The product was diluted with carrier and recrystallized from a hexane:methanol (1:4, v/v) mixture. The product was recovered by suction filtration and dried *in vacuo* at 35°C for four hours. The specific activity of the final product was 2.08 mCi/mmol. MP 135.5-137.5°C (corrected); radiochemical purity was determined to be greater than 98% by segmentation of tlc plates developed in the following systems: silica gel GF, ethyl acetate:glacial acetic acid (95:5, v/v, R_f 0.48) and silica gel GF, concentrated ammonium hydroxide:ethyl acetate:methanol (1:60:39, v/v, R_f 0.88). Radioactivity measurements were accomplished by liquid scintillation counting of 5 mm segments of the tlc plates. No discrete radiochemical impurities were detected. Treatment of a small sample with diazomethane followed by gas chromatography, using the conditions described above for 7a, showed the material to have a chemical purity greater than 98%. The infrared absorption spectrum, obtained as a Nujol® mull, exhibited maxima at 2851, 1607, 1528, 1343, 893 and 771 cm^{-1}. Unlabeled samples prepared during pilot runs were characterized by mass spectroscopy, ^{1}H NMR and elemental analysis, and compared fully to authentic reference material.

Dimethyl 2-(Tetradecyl-1-^{14}C)propanedioate (8)

Dimethyl malonate (13.7 mmol) was added to a solution of 13 mmol sodium methoxide dissolved in 6.6 mL methanol under a nitrogen gas atmosphere. The resulting mixture was stirred for 0.5 h at room temperature and then heated for

0.5 h at reflux temperature. The reaction was cooled to 20°C and 2.4 mmol (122.4 mCi) 1-bromotetradecane-1-^{14}C was added over a 10-min period. This mixture was heated at reflux temperature for 4.5 h and the solvent was evaporated on a rotary evaporator. The remaining solid was extracted into diethyl ether and washed with water. The organic layer was separated, dried over magnesium sulfate and filtered. The filtrate was concentrated on a rotary evaporator and excess dimethyl malonate was removed by fractional distillation *in vacuo*. The remaining oil crystallized on standing to give a 91.4% yield. The product was determined to be 85% pure by gas chromatography on a 2 m x 2 mm SE 30 on 100/120 mesh Gas Chrom Q glass column heated from 90-280°C at 16°/min using helium as the carrier gas and an FID (t_R 12.1 min); thin-layer chromatography: silica gel GF, hexane:diethyl ether:glacial acetic acid (85:15:1, v/v, R_f 0.53).

Monomethyl 2-(Tetradecyl-1-^{14}C)propanedioate (9)

A solution of 2.19 mmol of dimethyl 2-(tetradecyl-1-^{14}C)propanedioate (8), 3.11 mmol of 85% pure potassium hydroxide, 0.25 mL water and 10 mL methanol was heated at 45°C for eight hours and allowed to stir overnight at 40°C. The solvent was evaporated and the residue dissolved in 10 mL of 50% aqueous methanol. This mixture was washed (4x) with hexane (10 mL) and evaporated to dryness on a rotary evaporator. The residue was acidified to pH 1 with 6 N hydrochloric acid and the resulting precipitate extracted into ether. The organic layer was washed with saturated sodium chloride solution, dried over magnesium sulfate and filtered. The filtrate was evaporated to dryness and the product was recovered in an 89% yield after drying at 37°C *in vacuo* for four hours. Thin-layer chromatography was carried out in the following system: silica gel GF, hexane:diethyl ether:glacial acetic acid (84:15:1, v/v, R_f 0.23).

Methyl 2-Methylenehexadecanoate-3-^{14}C (6b)

Reaction of 1.94 mmol of monomethyl 2-(tetradecyl-1-^{14}C)propanedioate (9), according to a previously published procedure (1), gave 6b in an 85% yield. Gas chromatography using the conditions described above for compound 8 showed the material to have a 95% chemical purity (t_R 9.2 min); thin-layer chromato-

graphy:silica gel GF, hexane:diethyl ether (75:25, v/v, R_f 0.75).

Sodium Palmoxirate-3-^{14}C [Sodium 2-(Tetradecyl-1-^{14}C)oxiranecarboxylate, 1b]

Epoxidation of olefin 6b was carried out using the same procedure described for the carboxyl-labeled ester (7a) to give 7b in a 60% yield. The purified epoxide was hydrolyzed with sodium ethoxide in aqueous ethanol according to the procedure employed above and afforded the desired product in a 58% yield after recrystallization. The product had a specific activity of 51 mCi/mmol. Analysis by tlc-radioscan and gas chromatography of the methyl ester derivative, as described for the carboxyl-labeled sample, showed the material to have chemical and radiochemical purities greater than 98%. Unlabeled samples prepared during trial runs were fully characterized by ^{1}H NMR, infrared spectroscopy, mass spectroscopy and elemental analysis.

S-Palmoxirate-3-^{14}C-CoA Ester[S-[2-(Tetradecyl-1-^{14}C)oxiranecarboxylate] Coenzyme A, 2)]

Oxalyl chloride (1.1 mmol) was added to a suspension of 90 µmol sodium palmoxirate-3-^{14}C (1b) in 0.75 mL petroleum ether under a nitrogen atmosphere at 25°C. The mixture was stirred for one hour and the solvent and excess oxalyl chloride were removed in vacuo. A 0.5 mL aliquot of petroleum ether was added to the residue and after stirring for five minutes the reaction was concentrated to dryness under reduced pressure. A second 0.5 mL aliquot of petroleum ether was added to the reaction mixture and again the solvent was evaporated. The oily residue was dissolved in 1.5 mL dry THF and added dropwise over a five-minute period to a stirred mixture of 0.42 mmol sodium tetraborate decahydrate, 5.3 µmol coenzyme A, 0.15 mmol mercaptoacetic acid and 1.5 mL water at 25°C. The reaction was stirred for 0.5 h and 2 mL of 5% perchloric acid was added to the mixture. The organic solvent was evaporated under reduced pressure and the remaining suspension was centrifuged. The supernatant was decanted and the solid washed with 1 mL acetone followed by 1 mL diethyl ether. The solid was dissolved in 1.5 mL water and precipitated from solution by the addition of 5% perchloric acid to pH 2. The resulting suspension was centrifuged and the aqueous phase was decanted. The solid was washed with 0.5 mL acetone and then with 0.5 mL ether. The sample was suspended in 0.5 mL diethyl

ether and transferred to a vial. Evaporation of the solvent in vacuo provided a white crystalline solid. Typical yields for this procedure ranged between 40 and 79% (based on coenzyme A) for ten trial runs.

The specific activity of the labeled sample was 51 mCi/mmol based on the specific activity of the starting TDGA-^{14}C. The radiochemical purity of the product was estimated by HPLC analysis to be 90%. Chromatography was performed on a 4.6 x 250 mm Zorbax® octylsilane 10 μm column (Dupont, Wilmington, DE) eluted with a linear gradient starting with a solvent composition of 55% acetonitrile in 0.01 M tetrabutylammonium phosphate (adjusted to pH 5.5 with phosphoric acid) and increasing the concentration of acetonitrile to 83% over a five-minute period. The flow rate was 2 mL/min, the column temperature was 25°C and the effluent was monitored with a UV detector at a wavelength of 254 nm. In general, the chemical purities of material prepared during trial runs varied between 85 and 97% using the above HPLC system and were dependent, in part, on the purity of the starting coenzyme A. Purification of the crude TDGA-coenzyme A sample was readily accomplished by preparative chromatography on a reversed-phase octadecylsilane column using the following procedure: The TDGA-coenzyme A ester (2) was dissolved in water (4 mg/0.1 mL) and chromatographed on a 9.4 mm x 25 cm Whatman® Magnum 9, Partisil 10 μm ODS-3 column (Whatman Inc., Clifton, NJ). The column was eluted using a five-minute linear gradient formed from 50% acetonitrile in PIC® reagent A (tetrabutylammonium phosphate, Millipore, Waters Product Division, Milford, MA, adjusted to pH 6.0 with phosphoric acid) to 82.5% acetonitrile. The flow rate was 6 mL/min, column temperature 25°C and the effluent was monitored at a wavelength of 254 nm. The largest peak to elute (t_R 5.9 min) was collected and the acetonitrile evaporated in vacuo. The remaining aqueous solution was acidified to pH 2 with 1 N hydrochloric acid and the resulting suspension centrifuged. The supernatant was decanted and the solid was washed with 5 mL acetone followed by 5 mL ether. The white solid was dissolved in 0.4 mL water and centrifuged. The clear aqueous phase was removed and acidified with 5% perchloric acid to pH 2. The product was obtained by centrifugation and washed with 0.5 mL acetone followed by 0.5 mL diethyl ether. The white crystalline

solid was recovered in a 32% yield after drying in vacuo over phosphorous pentoxide. HPLC analysis using the system described above showed the material to be a single component with no impurities being detected. Chemical and spectroscopic analysis of an unlabeled sample, prepared during trial runs, were in full agreement with the structure of the S-TDGA-coenzyme A thioester (2). Elemental Analysis: Calcd. for $C_{38}H_{66}N_7O_{18}P_3S \cdot 2H_2O$ (1070.00): C, 42.66; H, 6.59; N, 9.16; Found: C, 42.44; H, 6.48; N, 9.08.

Mass Spectrum: Fast Atom Bombardment (FAB) mass spectroscopy, in a glycerol matrix, gave a positive ion spectrum with a protonated molecular ion corresponding to m/z 1034 $(M+H)^+$.

Proton NMR Spectrum: The 360 MHz proton NMR spectrum exhibited the appropriate resonances for the combination of coenzyme A and TDGA.

Ultraviolet Absorption Spectrum: The compound exhibited an absorption maximum at 256 nm ($\varepsilon = 1.40 \times 10^4$ M^{-1} cm^{-1}) and a minimum at 225 nm ($\varepsilon = 4.56 \times 10^3$ M^{-1} cm^{-1}, 1 mg/100 mL water).

Infrared Spectroscopy: The absorption spectrum of a sample prepared in a potassium bromide pellet exhibited major bands at 2930, 1693, 1230, 1050, 952 and 500 cm^{-1}.

Chemical Analysis: The product gave a negative nitroprusside test (18) for free sulfhydryl groups. The test was positive when 2 was hydrolyzed with 0.2 M sodium hydroxide in methanol for 2 min, indicating cleavage of the thioester bond. Acidification and extraction of the hydrolysis mixture with ethyl acetate followed by evaporation of the solvent provided the freed TDGA-^{14}C. Treatment of the acid with diazomethane in diethyl ether and gas chromatography of the resulting methyl ester, as described above, showed the material to be greater than 99% pure with no epoxide-ring-opened products being detected. In addition, the material cochromatographed with an authentic sample of methyl TDGA.

STABILITY

The radiochemical purity of a sample of sodium TDGA-1-^{14}C, with a specific activity of 2.08 mCi/mmol, decreased approximately 4% over a one-year per-

iod when stored as a dry solid at -20°C. A sample of the methyl ester (7a), having a specific activity of 8.3 mCi/mmol, exhibited a much faster rate of radiolytic self-decomposition. In this case, material with an initial radiochemical purity of greater than 99% was determined to be only 86% pure after five months of storage at -20°C. Seven discrete radiochemical impurities were detected in the sample by tlc-radioscan. The rate of decomposition was approximately 4% per year for a sample of methyl TDGA labeled in the hydrocarbon chain (7b) with a specific activity of 9.65 mCi/mmol. The rate decreased to 2% per year when the specific activity was 3.26 mCi/mmol. An unlabeled sample stored at room temperature during the same period exhibited no decomposition.

The carbon-14-labeled TDGA-CoA ester (2) was found to be most stable when stored refrigerated (-20°C) as the dry solid. A sample stored in this manner underwent approximately 3-4% decomposition per month. The radiochemical purity of a sample dissolved in water (10^{-4} M) and stored frozen at -78°C decreased at a rate of about 8-10% per month.

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