Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXV, No. 6

(ALL Z)-4,7,10,13-NONADECATETRAEN-1-OL 4-METHYLBENZENE SULFONATE: PREPARATION AND USE IN A ONE-STEP SYNTHESIS OF ARACHIDONIC ACID LABELED WITH CARBON-14

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SUMMARY

A key precursor to carbon-14 labeled arachidonic acid, namely (all Z)-4,7,10,13-nonadecatetraen-1-ol 4-methylbenzene sulfonate, was synthesized in 7 steps from 1-heptyne, 1,4-dichloro-2-butyne and propargyl alcohol and it was purified to be free of other isomers. The availability of this compound, which can be successfully stored, allows the repeated preparation of arachidonic-1-^{14}C acid in isotopically and chemically pure form by a simple one-step carbonation reaction of the readily prepared corresponding bromide. The labeled arachidonic acid is obtained at 100% chemical and radiochemical purity.

Key Words: (All Z)-4,7,10,13-Nonadecatetraen-1-ol 4-methyl benzene sulfonate, arachidonic-1-^{14}C acid

Arachidonic acid labeled with ^{13}C or ^{14}C, as the methyl ester, has been reported in the literature.[1-3] It was prepared by the partial (Lindlar) reduction of 4,7,10,13-nonadecatetrayn-1-ol followed by conversion to the corresponding mesylate. The isolated mesylate was heated with labeled KCN followed by hydrolysis in methanol to give labeled methyl arachidonate. Because the trans isomer, over-reduced products, and the small amounts of compounds with different degrees of unsaturation were also formed in the Lindlar reduction process, labeled methyl arachidonate prepared in this manner must be purified by preparative thin layer chromatography (tlc) and by argentation tlc. Labeled arachidonic acid is best prepared in small

0362–4803/88/060635–09$05.00

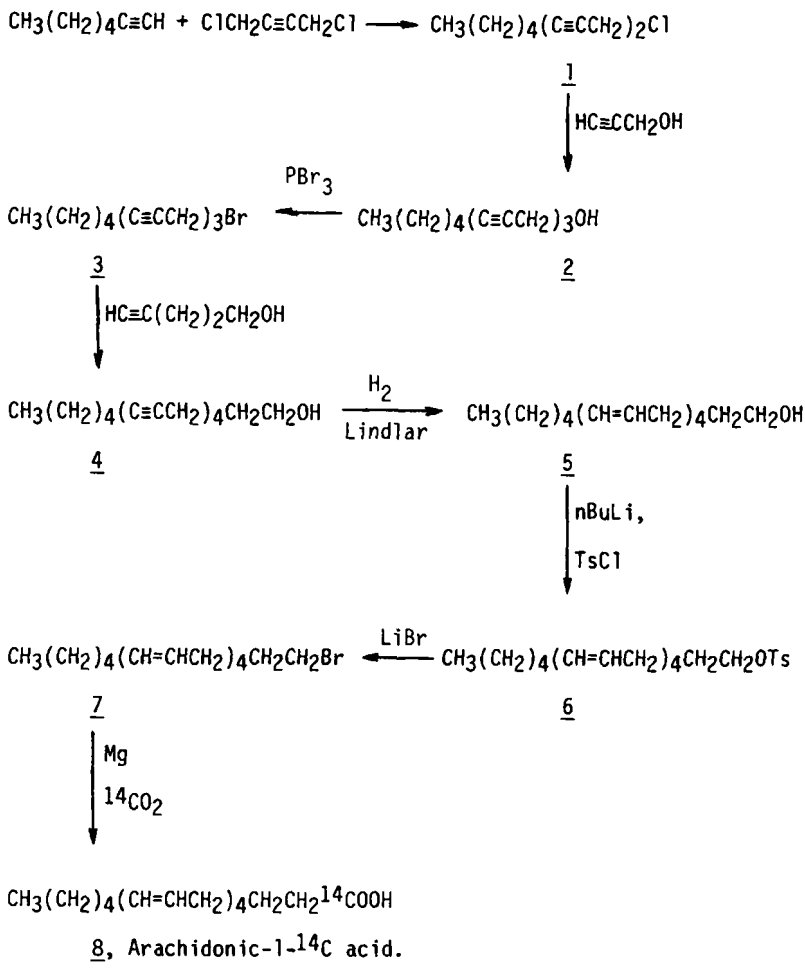
Received September 1, 1987
Revised October 12, 1987

quantities for short-term use because of its tendency to undergo chemical and radiolytic decomposition. The routine use of arachidonic-1-^{14}C acid in various biological studies requires a continuing supply of pure material and this led us to develop a more convenient and reliable method for the repeated preparation of this important labeled substance.

As reported by Rachlin,[4] arachidonic acid could be prepared by a one-step carbonation of 1-chloro-4,7,10,13-nonadecatetraene which was prepared by a number of steps involving unstable intermediates.[5] To avoid going through the entire synthetic sequence every time and to eliminate the tedious purification of the final product, it was desirable to have available a precursor which not only can be obtained free from other isomers but is also stable enough to be stored for future use as the need arises. In addition, the precursor should be easily converted to the corresponding halide under mild conditions without scrambling the double bonds. (All Z)-4,7,10,13-Nonadecatetraen-1-ol 4-methylbenzene sulfonate[6] was selected as the precursor since it satisfies all of these requirements.

As shown in the scheme, compound 5 was prepared in several steps from 1-heptyne and 1,4-dichloro-2-butyne according to literature procedures, with some modification. In this sequence, we found that compound 3 readily decomposed while in contact with water. During our work-up, we purified the reaction mixture quickly on a silica gel column to avoid water and obtained 3, in better quality, and converted it to 4 immediately after isolation. The yield of 4 was reduced substantially when 3 was stored for some time before use. The hydrogenation rate of 4 to 5 over Lindlar Vitamin A catalyst varied from batch to batch due to the poisoning effect from the small amount of impurities present in 4. To ensure the completion of hydrogenation, the reaction was monitored not only by the hydrogen uptake but also by HPLC analysis on a Dupont Zorbax ODS semipreparative reversed phase column. The tosylation of 5 directly with tosyl chloride in pyridine provided very little product. However, with the use of n-butyl lithium the tosylation proceeded as desired. Although the reaction product appeared to be a single component when examined by tlc on silica gel after purification

Scheme. Preparation of Arachidonic-1-^{14}C Acid.



8, Arachidonic-1-^{14}C acid.

on a silica gel column, the trans isomer and other compounds with different degrees of unsaturation were present as impurities and had to be removed by an additional column chromatography on silica gel impregnated with 15% silver nitrate. The tosylate 6 can be stored at -80°C under argon for extended periods of time. The bromination of 6 was accomplished by refluxing with lithium bromide in acetone and the Grignard carbonation of the resulting 7 with $^{14}CO_2$ gave arachidonic-1-^{14}C acid in one radiochemical step. A simple purification on a silica gel column was sufficient to provide labeled arachidonic acid in pure form as shown by tlc on silica gel

impregnated with silver nitrate and by HPLC analysis on a Dupont Zorbax ODS semipreparative reversed phase column (Fig. 1). Commercially available arachidonic acid as well as that prepared by literature methods has been reported to show UV absorption at 230-260 nm with a molar extinction coefficient of 10^3 to 10^4.(6,7) Our product showed no UV absorption by the UV detector at 260 nm as seen in Fig. 1.

Radiochemical yield for the carbonation procedure is 36% of purified product based on carbon-14 dioxide. The chemical yield is 71% based on conversion of the tosylate 6 to labeled arachidonic acid.

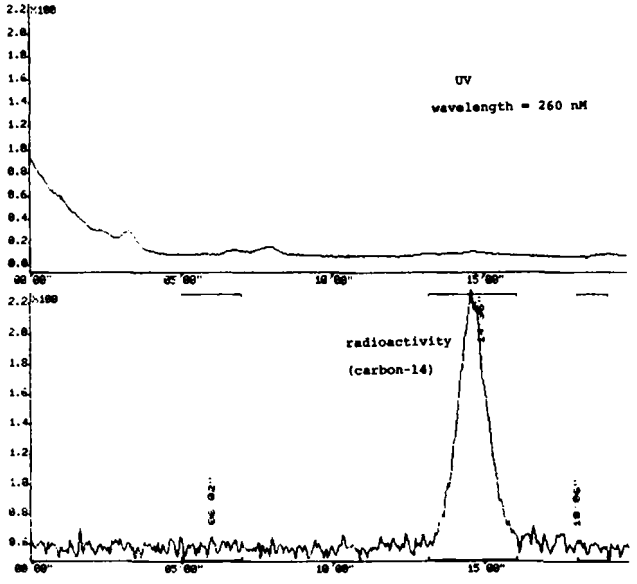


Fig. 1. UV and radiochromatogram of arachidonic-1-^{14}C acid by HPLC.

EXPERIMENTAL

General. Tetrahydrofuran (THF) was distilled from sodium ribbon using benzophenone as an indicator.

Spectra were recorded on standard instruments by the staff of the Physical Chemistry Department and microanalyses were performed by the

Microchemical Laboratory, both of Hoffmann-La Roche Inc. Radiochemical purity was determined on thin layer chromatograms with a Packard Model 7201 Radiochromatogram Scanner System, and by HPLC on a Dupont Zorbax ODS semipreparative reversed phase column with a Raytest Ramona-D (in/us corporation) flow-through monitor using the triple trace radiochromatographic software system. Radioactivity was measured by the liquid scintillation technique with a Packard Tricarb Model 2010 spectrometer.

1-Chloro-2,5-undecadiyne (1). To 1.15 M of ethyl magnesium bromide in 290 mL of dry THF was added slowly a solution of 95 g (0.99 M) of 1-heptyne in 70 mL of THF. The resulting mixture was stirred under nitrogen at room temperature overnight after which time 6.8 g of cuprous chloride was added and stirring continued for another five minutes. After cooling in an ice-bath, 385 g (3.12 M) of 1,4-dichloro-2-butyne was added rapidly and the reaction mixture was heated at 40°C for 16 hours. After cooling, 6N sulfuric acid was added and the product was extracted with ether. The ether extract was washed twice with saturated ammonium chloride, then twice with water, and finally with brine. After drying over sodium sulfate, the ether was evaporated and the residue was vacuum distilled. Recovered 1,4-dichloro-2-butyne was distilled below 75°C and 106 g of 1 (0.58 M, 58.6%) was collected at 90-120°C as a pale yellow liquid.

2,5,8-Tetradecatriyne-1-ol (2). To a flask containing 1.18 M of ethyl magnesium bromide in 770 mL of dry THF cooled in a water bath was added 31.4 g (0.595 M) of propargyl alcohol. After refluxing for 75 min. under nitrogen, 6.6 g of cuprous chloride was added and refluxing was continued for another 15 min. after which time a solution of 0.58 M of 1 in 420 mL of THF was added to the cooled reaction mixture. Refluxing was continued for an additional 68 hours. Dilute sulfuric acid was then added and the product was extracted with ether. After washing the extract with aqueous ammonium chloride, water, brine and drying over sodium sulfate, the ether was removed by evaporation. The residual product was purified by crystallization from hexane at -5°C. A total of 69.7 g (0.342 M, 58%) was obtained with a purity

of greater than 95% as shown by gas chromatography (3% SE 30 4 ft. column at 140°C with a retention time of 11 min.).

1-Bromo-2,5,8-tetradecatriyne (3). To a solution of 17.64 g (87.3 mM) of 2 in 80 mL of anhydrous ether containing 0.4 mL of dry pyridine was added slowly 2.9 mL (30.8 mM) of phosphorous tribromide. The resulting reaction mixture was refluxed under nitrogen for 4 hours. After cooling and filtration, the filtrate was concentrated and quickly purified on a silica gel (70-230 mesh) column (2 in. x 20 in.) packed in hexane. The column was eluted with hexane changing to hexane-methylene chloride (7:3) as the chromatography progressed. A dark brown impurity stayed on the column and 16.92 g (63.8 mM) of relatively pure product was obtained which was used for the next step immediately after isolation. This compound showed one major component at Rf 0.8 by tlc on a silica gel plate with hexane-methylene chloride (1:1) as the eluting solvent. The compound was detected by 0.05% potassium permanganate spray. The chemical yield was 73%.

4,7,10,13-Nonadecatetrayn-1-ol (4). A solution of ethyl magnesium bromide (180 mM) in 200 mL of anhydrous THF was treated with the slow-addition of a solution of 7.14 g (85 mM) of 4-pentyn-1-ol[8] in 50 mL of anhydrous THF. The resulting mixture was refluxed for 45 minutes under nitrogen and then 1.88 g of cuprous chloride was added. After refluxing for 20 minutes, a solution of 16.92 g of 3 in 50 mL of anhydrous THF was added rapidly to the cooled reaction mixture and refluxing was then continued for another 16 hours. The reaction mixture was quenched with the addition of aqueous ammonium chloride solution and the product was extracted into ether. After washing the extract with water and brine, the ether layer was dried over magnesium sulfate and then concentrated to a brown solid which was purified on a silica gel column eluting with 1% methanol in methylene chloride. A total of 15.6 g (58.2 mM) of 4 was isolated (91% yield). We noticed some polymerization of this compound upon evaporation or while in contact with air. The yield of 4 was substantially reduced when 3 was stored and not used immediately after isolation. This product 4 was used for the next step without further purification.

All-cis-4,7,10,13-nonadecatetraen-1-ol (5). A solution of 19.1 g of 4 (0.07 M) in 150 mL of ethanol containing 0.4 mL of quinoline and 4 g of Lindlar catalyst (9) was reduced under 45 psi of hydrogen until the absorption stopped. After removing the catalyst by filtration, the reaction mixture was analyzed by HPLC on a Dupont Zorbax ODS semipreparative reversed phase column for completion of the reduction. At the flow rate of 2 mL/min. with methanol, the retention time of 4 was 7.2 min. and the retention time of 5 was 9.2 min. Fresh catalyst was added and the hydrogenation process was repeated two more times when the HPLC chromatogram showed only one major product peak and the hydrogenation uptake was close to the theoretical amount. After removing the catalyst, the residue obtained after evaporation was purified on a silica gel column eluting with 1% methanol in methylene chloride. A total of 21.7 g (0.078 mM, 109%) of product 5 was obtained.

All-cis-4,7,10,13-nonadecatetraen-1-ol 4-methylbenzene sulfonate (6). A solution of 6.1 g (22.1 mM) of 5 in 60 mL of THF was treated at 0°C with 11.6 mL of n-BuLi (2.3 M, 26.6 mM) until the solution darkened. The mixture was stirred at room temperature for half an hour longer and 5.5 g (29 mM) of tosyl chloride in 25 mL of THF was added. This was stirred at room temperature overnight and then poured into ice water. The product was extracted into ether which was dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on a silica gel column eluting with hexane-methylene chloride (1:1) and 5.71 g (13.3 mM, 59.9%) of crude product was isolated. This was again purified on a silica gel column impregnated with 15% of silver nitrate eluting gradiently with methylene chloride to methylene chloride-ethyl acetate 60:40 (v/v), followed by elution over a silica gel column with hexane-methylene chloride (1:1) to remove the inorganic salt washed out from the first column to yield 2.75 g (6.4 mM, 29%) of pure tosylate as an oil; ir (3% $CHCl_3$ solution): 2960, 2930, 1303, 1178 cm^{-1}; nmr ($CDCl_3$) δ : 0.89 (t, 3H, CH_3), 1.28 (m, 8H, $-CH_2-$), 1.6-2,2 (m, 4H, CH_2), 2.46 (S, 3H, Ar-CH_3), 2.79 (m, 6H, =CH_2=), 4.06 (t, 2H, OCH_2), 5.36 (m, 8H, $CH=CH-$), 7.44 and 7.80 (q, 4H, Ar); ms m/e (relative intensity) 91 (100), 155, 172, 258, 373, 359, 430; Anal. Calcd.

for $C_{26}H_{38}O_3S$: C, 72.52; H, 8.89; S, 7.44. Found: C, 71.24; H, 8.38; S, 7.27. The purity was analyzed by HPLC on a Dupont Zorbax ODS column. The retention time was 19.5 min at a flow rate of 3 mL/min. using 10% water in methanol. The product showed only one component on a silica gel tlc plate impregnated with 10% $AgNO_3$. The plate was eluted with CH_2Cl_2-EtOAc (6:4).

All-cis-1-Bromo-4,7,10,13-nonadecatetraene (7). A mixture of 340 mg (0.79 mM) of 6 and 220 mg (2.53 mM) of LiBr in 20 mL of dry acetone (distilled from potassium permanganate) was refluxed for two hours. After cooling and filtration, the filtrate was concentrated and chromatographed on a silica gel column with hexane. A quantity of 264 mg (0.78 mM, 98%) of 7 was obtained as a colorless liquid, ir (3% $CHCl_3$ solution): 2965, 2935, 1650 cm^{-1}; nmr ($CDCl_3$) δ : 0.89 (t, 3H, CH_3), 1.3 (m, 6H, CH_2), 1.8-2.4 (m, 6H, CH_2 and CH_2), 2.85 (m, 6H, CH_2), 3.42 (t, 2H, CH_2 Br), 5.38 (m, 8H, -CH=CH-); ms m/e 186, 200, 240, 267, 338.

Arachidonic-1-^{14}C acid (8). To 264 mg (0.78 mM) of 7 and 18.9 mg (0.78 mM) of Mg in 0.5 mL of anhydrous THF was added 0.86 mL of 0.9 N freshly prepared THF solution of ethyl magnesium bromide. The mixture was refluxed for 3 hours under argon. Carbon-14 dioxide was generated from 312 mg (1.56 mM, 85.8 mCi) of $Ba^{14}CO_3$, specific activity of 55 mCi/mM, and vacuum transferred into the solution. After stirring overnight at room temperature, volatile components were removed under vacuum and the residue was treated with 1N hydrochloric acid. The resulting solution was extracted with ether and the dried ether layer was concentrated under vacuum to remove solvent and any propionic-1-^{14}C acid. The residue was purified on a silica gel column eluting with $CHCl_3$-EtOAc gradiently from 0 to 40%. A total of 31 mCi (36% radiochemical yield) of arachidonic-1-^{14}C acid (170 mg, 0.56 mM) with specific activity of 55 mCi/mM was obtained. This product was pure as shown by tlc on silica gel with $CHCl_3$-EtOAc-EtOH (7:3:0.2%) and on silica gel impregnated with 10% silver nitrate with hexane-EtOAc acetic acid (60:40:3). It showed only one radioactive component by HPLC analysis on a Dupont Zorbax ODS column, with methanol, at a flow rate of 1 mL/min, with retention time of 14 min. 36 sec. The compound was detected by both

radioactivity and UV at 260 nm (Fig. 1). The radiochemical purity was found to be 100% after background subtraction calculated by the triple trace radiochromatographic software system.

Acknowledgment

We thank Dr. J. Blount and his staff in our Physical Chemistry Department, in particular, Dr. W. Benz for mass spectra, Dr. F. Scheidl for microanalyses and Dr. T. Williams for nmr spectra.

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