SYNTHESIS OF [^{14}C]-LABELLED EICOSA-5,8,11-TRIYNOIC ACID AND CONVERSION TO ANTI-INFLAMMATORY AMIDES

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SUMMARY

A four step synthesis of [5,6-^{14}C]-eicosa-5,8,11-triynoic acid (1) from [^{14}C]-labelled acetylene is described. [$^{14}C_2$]-acetylene was converted to 5-chloro-[1,2-^{14}C]-pentyne via reaction of its monolithium salt with 3-bromo-1-chloropropane. The doubly labelled 5-chloropentyne thus obtained was transformed to [5,6-^{14}C]-hex-5-ynoic acid which was then coupled with 1-chloro-tetradeca-2,5-diyne to give the title compound. Using 2-(2-aminoethoxy)ethanol and 1-(2-hydroxyethyl)piperazine, amides (2) and (3), which had previously been found to be potent inhibitors of the 5-lipoxygenase enzyme, were prepared from [^{14}C]-labelled eicosatriynoic acid by way of acylimidazole chemistry.

Keywords: polyunsaturated fatty acid, lipoxygenase, anti-inflammatory, lithium [$^{14}C_2$]-acetylide, [5,6-^{14}C]-eicosa-5,8,11-triynoic acid, CD 554, CD 581.

$$CH_3\text{-}(CH_2)_7\text{-}C{\equiv}C\text{-}CH_2\text{-}C{\equiv}C\text{-}CH_2\text{-}\overset{*}{C}{\equiv}\overset{*}{C}\text{-}(CH_2)_3COR$$

1 R = OH

2 R = -NH-$(CH_2)_2$-O-$(CH_2)_2$-OH

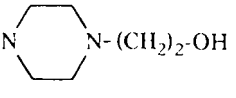
3 R = 

Figure 1

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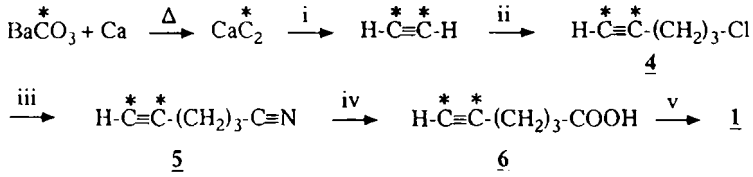
INTRODUCTION

Phospholipid bound arachidonic acid (all-cis eicosa-5,8,11,14-tetraenoic acid) is a constituent of the plasma membrane of eucaryotic cells. Release of the free acid form by phospholipases, followed by enzyme catalysed oxidation gives rise to mediators of inflammation and hypersensitivity: arachidonic acid is transformed by cyclo-oxygenase to prostaglandins (1) and by lipoxygenases to pro-inflammatory hydroxy-eicosatetraenoic acids (HETES) and leukotrienes (2). Many anti-inflammatory drugs are inhibitors of cyclo-oxygenase or of both cyclo-oxygenase and lipoxygenase, but these compounds have little or no beneficial effect in topical inflammation. Since HETES and leukotrienes have been especially implicated in inflammatory conditions of the skin (3), one approach to new therapies applicable in dermatology has been to search for *selective* inhibitors of lipoxygenase. Eicosa-5,8,11-triynoic acid, a substrate analogue of arachidonic acid, is a selective inhibitor of leukotriene biosynthesis in vitro (4). In our laboratories, chemical modification of the carboxylic acid function of eicosa-5,8,11-triynoic acid has led to compounds with increased biological potency, active in vivo; in particular, N-[2-(2-hydroxyethoxy)ethyl]eicosa-5,8,11-triynoic amide (2) and 1-(eicosa-5,8,11-triynoyl)-4-(2-hydroxyethyl)piperazine (3) have been found to possess properties consistent with good topical anti-inflammatory activity (5). [^{14}C]-labelled 2 and 3 were required for skin penetration studies. Of additional interest, 1 may be used as a precursor for the synthesis of labelled eicosa-5,8,11-eicosatrienoic acid (6,7).



(i) H_2O

(ii) n-BuLi / HMPA / THF, -78°C / Br-$(CH_2)_3$-Cl

(iii) NaCN / DMSO, 70°C, 20 min.

(iv) KOH 6N, 75°C, 5h. / H^+

(v) CH_3-$(CH_2)_7$-C≡C-CH_2-C≡C-CH_2Cl / n-C_3H_7-MgCl / CuCN / THF, 50°C, 3h.

Figure 2

RESULTS AND DISCUSSION

The synthetic route is shown in Figure 2. Labelled acetylene was prepared by hydrolysis of calcium [^{14}C]-carbide, obtained by fusing barium [^{14}C]-carbonate with excess calcium, and trapped as a solid in a liquid nitrogen cooled trap (8,9,10). Several methods of mono-alkylating acetylene were tried, with variable results. Preparation of sodium acetylide using sodamide in liquid ammonia (11) or in tetrahydrofuran (12) followed by the addition of 1-bromo-3-chloropropane gave only very low yields of 5-chloropentyne (4); attempts to prepare and alkylate lithium acetylide in dimethyl sulfoxide (13) were not successful. Best results (60% yield of 4) were obtained when monolithium acetylide was prepared using n-butyllithium in tetrahydrofuran with hexamethylphosphoramide as co-solvent (14,15). Reaction of 4 with sodium cyanide in dimethyl sulfoxide followed by hydrolysis of the product (5) gave [5,6-^{14}C]-hex-5-ynoic acid (6).

For Cu(I) catalysed coupling with 1-chloro-tetradeca-2,5-diyne, the chloromagnesium acetylide was prepared from 6 using n-propylmagnesium chloride. The exothermicity of the coupling reaction was much greater when an alkylmagnesium bromide was used, and yields were lower; indeed, in some exploratory experiments using unlabelled 5-hexynoic acid, with bromide rather than chloride as the alkylmagnesium counterion, reactions were uncontrollably exothermic, and resulted in complex mixtures.

Conversion of eicosa-5,8,11-triynoic acid to amides 2 and 3 was studied first using the acid chloride and mixed-anhydride methods. These methods gave inferior results when compared to amide formation using carbonyldiimidazole, with respect to both yield and ease of purification.

EXPERIMENTAL

Instrumentation

Radioactivity determinations were carried out by liquid scintillation counting using a Beckman LS 2800 instrument with Picofluor 30 (Packard) as the scintillation cocktail. Radiochemical purities were determined by TLC on Merck 60F$_{254}$ plates using a Berthold Automatic TLC Linear Analyser, and by HPLC on normal phase (DuPont Zorbax SIL) or

reverse phase (DuPont Zorbax ODS) columns using an LDC Milton Roy instrument equipped with a Berthold LB 5034 splitter/mixer and a Berthold LB 503 monitor for liquid scintillation detection. Data acquisition and treatment were effected by a Berthold LB 510 system. UV spectra were recorded on a Beckman 25 spectrophotometer. Mass spectra were obtained using a Nermag R10-10C.

Chemicals

Barium [^{14}C]-carbonate (specific activity 56 mCi/mmole) was obtained from the CEA, Saclay, France. All solvents were dried before use; tetrahydrofuran was distilled from sodium-benzophenone ketyl. Solutions of reaction products were dried over anhydrous magnesium sulfate.

[$^{14}C_2$]-Acetylene

An intimate mixture of $BaCO_3$ (1.97 g, 10 mmol), [^{14}C]-$BaCO_3$ (1.97 g, 10 mmol) and granular (0.5-0.8 mm) calcium (9.5 g, 237 mmol) was placed in two Pyrex tubes and covered with a 5 mm layer of calcium. The tubes were purged with argon and then heated to incandescence with an oxy-butane torch. The calcium carbide thus formed was hydrolysed in water (150 ml) and the exit gases were passed through a cold water condenser and phosphorus pentoxide drying tube to a vacuum line. The labelled acetylene was condensed in a double glass coil cooled in liquid nitrogen.

5-Chloro-[1,2-^{14}C]-pentyne (4)

The acetylene was transferred by bulb to bulb distillation into a three neck reaction flask containing anhydrous tetrahydrofuran (15 ml) cooled in liquid nitrogen. The reaction vessel was then warmed to -78° C, stirred for 15 minutes, then placed under an argon atmosphere. A 1.6 M hexane solution of n-butyllithium (5 ml, 8 mmol) was introduced dropwise over 15 minutes. The solution was stirred for 30 minutes before adding a solution of 1-bromo-3-chloropropane (0.79 ml, 8 mmol) in hexamethylphosphoramide (9 ml). Stirring was continued for 30 minutes at -78°C, and then for a further 60 minutes at room temperature. The reaction solution was poured into brine (100 ml) under a layer of diethyl ether (100 ml). Final workup, purifying by chromatography on a short silica gel column eluted with ether, gave 335 mCi of 5-chloro-[1,2-^{14}C]-pentyne which co-

chromatographed on TLC (silica gel; 2:3 CH_2Cl_2:n-hexane; rf 0.8) with authentic unlabelled 4. The yield was 60% with respect to $BaCO_3$.

5-Cyano-[1,2-^{14}C]-pentyne (5)

A solution of sodium cyanide (0.318 g, 6.5 mmol) in dimethyl sulfoxide (4 ml) was heated and stirred at 50°C. A solution of 4 (335 mCi) in the same solvent (2 ml) was added slowly so that the reaction temperature remained below 70°C. The reaction mixture was heated at 70°C for 20 minutes after the addition, then cooled and poured into saturated ammonium chloride/diethyl ether. Flash chromatography on silica gel eluted with methylene chloride gave 5-cyano-[1,2-^{14}C]-le
pentyne (202 mCi; 61% yield) which co-chromatographed with unlabelled **5** (silica gel; CH_2Cl_2; rf 0.75).

[5,6-^{14}C]-Hex-5-ynoic acid (6)

A mixture of 5 (202 mCi) and 6N potassium hydroxide (4.2 ml) was heated at 75°C for 5 hours, then cooled and acidified to pH 2 with 6N HCl. Extraction and chromatography on a silica gel column eluted with diethyl ether gave [5,6-^{14}C]-hex-5-ynoic acid (155 mCi; 77% yield) which co-chromatographed with unlabelled 6 (silica gel; 9:1 CH_2Cl_2:acetone; rf 0.55).

[5,6-^{14}C]-Eicosa-5,8,11-triynoic acid (1)

1-Propylmagnesium chloride was prepared in tetrahydrofuran (190 ml) at reflux, from 1-chloropropane (43.5 ml, 0.5 mol) and magnesium turnings (12.2 g, 0.5 mol). The solution thus obtained was filtered under nitrogen pressure and titrated against a 0.5M solution of 2-butanol in toluene, using 1,10-phenanthroline as indicator.

A sample (3.24 ml, 6 mmol) of the propylmagnesium chloride solution was transferred to a reaction flask under argon and cooled to 0°C. A tetrahydrofuran solution (0.4 ml) of 6 was introduced slowly. An exothermic reaction ensued, accompanied by the evolution of propane. When gas evolution subsided, the solution was heated at 50°C for 15 minutes. Copper(I) cyanide (18 mg, 0.2 mmol) was added, followed 15 min. later by 1-chlorotetradeca-2,5-diyne (0.45 g, 2 mmol) dissolved in tetrahydrofuran (0.9 ml). Heating

at 50°C was continued for 3 hours. The cooled reaction mixture was poured into 3N HCl under a layer of ethyl acetate (100 ml). The crude product was purified by column chromatography on silica gel eluted with 93:7 methylene chloride:acetone. [5,6-^{14}C]-Eicosa-5,8,11-triynoic acid (54 mCi; 35% yield) was obtained as a beige solid which co-chromatographed with authentic unlabelled 1 (silica gel; 9:1 methylene chloride:acetone; rf 0.63).

N-[2-(2-Hydroxyethoxy)ethyl]-[5,6-^{14}C]-eicosa-5,8,11-triynoic amide (2)

Working in an argon-filled glove box, 1,1'-carbonyldiimidazole (0.21 g, 1.3 mmol) was added to a solution of 1 (54 mCi) in methylene chloride (4 ml) at room temperature. After 1 hour the solution was cooled to 0°C and 2-(2-aminoethoxy)ethanol (0.21 g, 2 mmol) in methylene chloride (1 ml) was added. After a reaction time of 2 hours, 2N HCl (3 ml) was added. The crude product was purified by flash chromatography on silica gel eluted with 7:3 methylene chloride:acetone. The pure 2 so obtained (36 mCi; 67 % yield) co-chromatographed with unlabelled N-[2-(2-hydroxyethoxy)ethyl]eicosa-5,8,11-triynoic amide and the UV spectra were identical.

1-[5,6-^{14}C]-(Eicosa-5,8,11-triynoyl)-4-(2-hydroxyethyl)piperazine (3)

With the operating conditions used for the preparation of (2), 1 (3.6 mCi), 1,1'-carbonyldiimidazole (0.21 g, 1.3 mmol) and 1-[2-(2-hydroxyethoxy)ethyl]piperazine (0.16 g, 0.124 mmol) gave pure 3 (1,5 mCi; 79% yield) after chromatography on silica gel using 7:3 methylene chloride:methanol as eluant. This compound had a UV spectrum identical to that of authentic unlabelled 3, and co-chromatographed with it.

Radiochemical Purities of 2 and 3

By TLC (silica gel, 7:3 methylene chloride: acetone) the radiochemical purities of both 2 and 3 were >98%.

By HPLC, the radiochemical purity of 2 (Zorbax ODS, 85:15 methanol:water) was >97%, and of 3 (Zorbax ODS, 75:25:0.4 acetonitrile:water:trifluoroacetic acid) was >96%.

The specific activities of 2 and 3 were 45.9 mCi/mmol as determined by UV, and 50.2 mCi/mmol as determined by their mass spectra.

Decomposition of 2 and 3 was considerably reduced by the presence of water; these compounds were best conserved by storage in solution in 9:1 ethanol:water, under nitrogen.

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   (b) Compounds 2 and 3 (unlabelled) bear CIRD Galderma codes CD 554 and CD 581.
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