PREPARATION OF METHYL [1-^{14}C]DOCOSA-7,10,13,16-TETRAENOATE

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SUMMARY

Methyl [1-^{14}C]docosa-7,10,13,16-tetraenoate was prepared from [1-^{14}C]arachidonic acid by two successive Arndt-Eistert syntheses which also can be used for the step-wise homologation of other labeled fatty acids. After purification by gas chromatography the specific activity was 6.16 mCi/mmol, and the radiochemical purity was 97%.

INTRODUCTION

All-*cis*-C_{22}-polyenoic acids (1-3) derived from linoleic acid (4) and/or α-linolenic acid (5) are important to the development of function of testicular tissue (1). Docosa-7,10,13,16-tetraenoic acid (1), docosa-4,7,10,13,16-pentaenoic acid (2), and docosa-4,7,10,13,16,19-hexaenoic acid (3) accumulate, respectively, in the rooster, rat, and human testes in large quantities during the time of sexual maturation (1,2). Numerous factors which adversely affect

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testicular development are associated with decreased levels of these fatty acids (3).

$CH_3(CH_2)_x(CH{=}CHCH_2)_y(CH_2)_zCO_2H$

1, x = 4; y = 4; z = 4
2, x = 4; y = 5; z = 1
3, x = 1; y = 6; z = 1
4, x = 4; y = 2; z = 6
5, x = 1; y = 3; z = 6
6, x = 4; y = 4; z = 2

The biosynthesis of the C_{22}-tetraenoic acid 1 and C_{22}-pentaenoic acid 2 from linoleic acid (4) proceeds by way of arachidonic acid (6) (2), also found in large quantities in rooster, rat, and human testes (1). The acid 1 is first formed from 6 and then dehydrogenated to 2. The latter has also been shown, using [5-^{14}C]-2, prepared in low specific activity by biosynthesis (4), to be converted to 6 by direct biohydrogenation and cleavage (4).

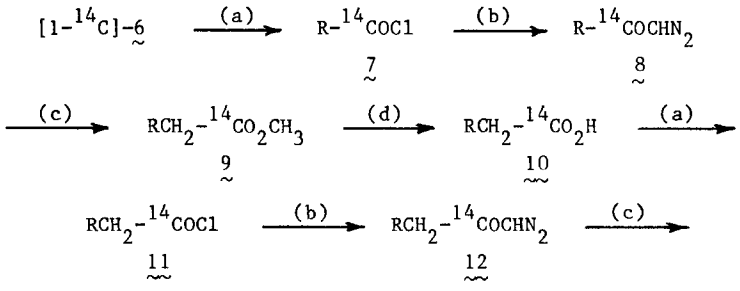
For continued study of their biosynthesis and further elucidation of their physiological role, carbon-14-labeled C_{22}-polyenoic acids or esters, depending on the mode of administration, of specific activity greater than 5 mCi/mmol are required.

Methyl [3-^{14}C]docosa-7,10,13,16-tetraenoate ([3-^{14}C]-1 methyl ester) with low specific activity (0.40 mCi/mmol) was prepared earlier from methyl [1-^{14}C]arachidonate ([1-^{14}C]-6 methyl ester) *via* a malonic ester synthesis (5). In a more efficient procedure we have now prepared [1-^{14}C]-1 methyl ester from [1-^{14}C]-6 in good yield by two successive Arndt-Eistert syntheses (Reaction Scheme) which can also be used for the step-wise homologation of other labeled fatty acids. The Arndt-Eistert synthesis has been previously applied to the large scale homologation of unsaturated carboxylic acids (6), including two polyunsaturated fatty acids (7).

RESULTS AND DISCUSSION

[1-^{14}C]Arachidonic acid ([1-^{14}C]-6, 50 μCi, 10.4 mCi/mmol) was converted to the acid chloride 7 with oxalyl chloride in benzene and to the diazo ketone 8

Reaction Scheme



[1-^{14}C]-1 methyl ester

R = $CH_3(CH_2)_4(CH{=}CHCH_2)_4CH_2CH_2$

(a) $(COCl)_2$/benzene. (b) CH_2N_2/ether. (c) $C_6H_5CO_2Ag$/Et_3N/MeOH. (d) KOH/H_2O/MeOH.

with diazomethane. Addition of silver benzoate in triethylamine to a methanolic solution of 8 effected a Wolff rearrangement (8) to 9. Analysis of the hexane-soluble product (41 μCi) by gas chromatography (GC) showed a small amount of [1-^{14}C]-6 methyl ester while 92% of the radioactivity had a retention time intermediate between those of 6 methyl ester and methyl docosa-7,10,13,16-tetraenoate (1 methyl ester) (Table I), the intermediate retention time consistent for that of methyl [1-^{14}C]heneicosa-6,9,12,15-tetraenoate (9).

Table I. Gas Chromatography Data for Fatty Acid Methyl Esters

Compound	Retention time, min[a]
6 methyl ester[b]	12.9
9[c]	14.7
1 methyl ester[b]	16.5

[a] Temperature programmed from 175 to 225 °C increasing at 5 °C/min. [b] Authentic sample. [c] Unlabeled.

Crude 9 was saponified with potassium hydroxide in aqueous methanol, and 10 was converted via the acid chloride 11 to the diazo ketone 12. Treatment of the crude reaction product with silver benzoate and triethylamine gave a mixture (28.3 μCi) which by GC had radioactivity corresponding to 3.6% [1-^{14}C]-6 methyl ester, 9.7% 9 and 77% [1-^{14}C]-1 methyl ester. Various unidentified substances with longer retention times than that of 1 methyl ester accounted for the remaining radioactivity. To aid in recovery, a small portion of 1 methyl ester was added, and [1-^{14}C]-1 methyl ester (17.8 μCi, 6.16 mCi/mmol) was isolated by preparative GC.

In preliminary synthetic experiments utilizing unlabeled arachidonic acid (6), products, identified as unlabeled 9 and 1 methyl ester by GC, when subjected to mass spectroscopy gave parent ions with m/z 332 and 346, respectively, consistent with their respective calculated molecular weights. During these synthetic experiments, the proton nuclear magnetic resonance (^{1}H NMR) spectra of the unlabeled intermediates 7-10 and 1 methyl ester were consistent with their assigned structures. These spectra showed that for each compound in the synthetic sequence there was some signal (Table II) well separated from

Table II. NMR Spectral Data of Polyene Fatty Acids and Derivatives

Compound	Proton	ppm[a]	Proton	ppm[a]
6[b]	CH_2CO_2	2.39	CO_2H	10.83[c]
7[b]	CH_2COCl	2.93		
8[b]	CH_2CO	2.38	$COCHN_2$	5.29
9[b]	CH_2CO_2	2.38	CO_2CH_3	3.78
10[b]	CH_2CO_2	2.40	CO_2H	9.62[c]
1 methyl ester	CH_2CO_2	2.37	CO_2CH_3	3.74

[a] Downfield from TMS in $CDCl_3$. [b] Unlabeled. [c] Variable.

neighboring signals which either was not present in the reactant or was present in the reactant but not in the product. This provided an easy method for

determining the success or failure of any given reaction. It was also evident from these spectra that the reaction mixtures remained remarkably free of impurities.

EXPERIMENTAL

Unless otherwise noted, reactions were run at room temperature and under dry nitrogen. Excess diazomethane was removed from reaction mixtures using a stream of dry nitrogen, and other evaporations were done under reduced pressure at or below room temperature. Benzene was purified and dried by distillation, and methanol was stored over Type 3A Linde Molecular Sieves. Triethylamine was boiled with acetic anhydride and distilled. The distillate was shaken with solid potassium hydroxide and redistilled. Analytical gas chromatography (GC) of unlabeled samples was done with a Varian 1520 Gas Chromatograph with an 8-ft x 1/8-in column. Radioactive samples were analyzed using a Varian 1800 Gas Chromatograph (radio-GC) with an 8-ft x 1/4-in column, and fractions were collected using a Packard Fraction Collector Model 850. The columns were packed with 10% SP 2340 on 100-120 mesh Supelcoport (Supelco, Inc.). The carrier gas was nitrogen at a flow-rate of 30 mL/min for the 1/8-in diameter column and helium at a flow-rate of 80 mL/min for the 1/4-in diameter column. The column temperature was programmed from 175 to 225 °C increasing at a rate of 5 °C/min. [1-^{14}C]-1 Methyl ester was isolated using an F and M Research Chromatograph with an 8-ft x 1/4-in column with packing as above. The carrier gas was helium at a flow-rate of 60 mL/min, and the temperature of the column was 190 °C. Fractions were collected in 10-cm tubes packed with glass wool. Radioactive samples were counted in a toluene-based scintillation fluid using a Packard Tricarb Spectrometer Model 3214 with a counting efficiency of 89%. The mass spectra were obtained using an LKB Model 9000 Gas Chromatograph/Mass Spectrometer with a 70 eV ionizing potential. Proton nuclear magnetic resonance (^{1}H NMR) spectra were observed with a JEOL JNM-MH-100 spectrometer.

Methyl [1-^{14}C]Docosa-7,10,13,16-tetraenoate ([1-^{14}C]-1 Methyl Ester). Arachidonic acid (11.9 mg, 39.1 μmol) was diluted to 10 mL with benzene, and 1.0 mL of this solution was combined with 0.27 mg (0.89 μmol) of [1-^{14}C]arachidonic acid (56.3 mCi/mmol). The resultant solution of [1-^{14}C]-6 (4.80 μmol, 50 μCi, 10.4 mCi/mmol) was evaporated, and the residue was stirred with benzene (0.5 mL) and oxalyl chloride (0.5 mL) for 15 min. The benzene and excess oxalyl chloride were evaporated and diazomethane, prepared from N-methyl-N-nitroso-p-toluenesulfonamide (0.43 g, 20 mmol), in alcohol-free ether (5-7 mL) was added. The mixture was stirred for 1 h, and the excess diazomethane was removed. The ether was evaporated, and the residue was dissolved in methanol (3 mL). To this solution was added silver benzoate (5 mg) in triethylamine (0.5 mL). The mixture was stirred for 1 h and then boiled for 15 min. The solvent was evaporated, and hexane (8 mL) was added to the residue. The mixture was stirred overnight, filtered through cotton and evaporated. The residue (41 μCi, 82%) was redissolved in hexane (5 mL). Analysis of an aliquot of this solution by radio-GC showed that methyl [1-^{14}C]heneicosa-6,9,12,15-tetraenoate (9) accounted for 92% of the radioactivity. The solvent was evaporated, and the residue was boiled with methanol (2.5 mL) and 10% aqueous potassium hydroxide (2.5 mL) for 1 h. The mixture was evaporated, acidified with 3 N hydrochloric acid (5 mL), and extracted with ether (3 x 10 mL). The ether extract was dried ($MgSO_4$), filtered and evaporated to give crude [1-^{14}C]heneicosa-6,9,12,15-tetraenoic acid (10). Crude 10 was treated with the same sequence of reactants in the same amounts as was [1-^{14}C]arachidonic acid ([1-^{14}C]-6) to give crude methyl [1-^{14}C]docosa-7,10,13,16-tetraenoate ([1-^{14}C]-1 methyl ester) (28.3 μCi, 57%). This crude product was dissolved in hexane (5 mL), and analysis of the solution by radio-GC showed that [1-^{14}C]-1 methyl ester accounted for 77% of the radioactivity. Unlabeled 1 methyl ester (0.50 mg, 2.9 μmol) was added. The resulting solution was subjected to preparative GC, and [1-^{14}C]-1 methyl ester (17.8 μCi, 6.16 mCi/mmol, 36% from [1-^{14}C]-6) was isolated with a radiochemical purity of 97% as determined by radio-GC.

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