SYNTHESIS OF 14-METHYL[2,3-^{3}H]HEXADECANOIC ACID

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SUMMARY

14-Methyl[2,3-^{3}H]hexadecanoic acid of a specific radioactivity of 51 Ci/mmol was prepared starting from 12-methyltetradecanoic acid isolated from wool fat. This fatty acid was converted into 12-methyltetradecanal which was reacted with ethyl diethylphosphonoacetate to yield 14-methylhexadecen-2-oic acid. The resulting compound was tritiated with gaseous $^3H_2$ in the presence of a 5% $Pd/BaSO_4$ catalyst. The reaction in dried dioxane gave products of a specific radioactivity corresponding to 87.9% of the theoretical value.

Key words: 14-Methylhexadecanoic acid, 12-Methyltetradecanoic acid, Cholesteryl 14-methylhexadecanoate, Catalytic tritiation

INTRODUCTION

Several papers from this laboratory demonstrated the importance of cholesteryl 14-methylhexadecanoate in protein synthesis [see (1,2) for reviews] and for the cancer growth (3). Since virtually nothing is known on the biosynthesis and metabolism of this ester and of its fatty acid moiety, the availability of 14-methylhexadecanoic acid with a high specific radioactivity suitable for metabolic experiments seemed to be desirable.

The catalytic reduction of unsaturated homologues seems to be a technique generally useful for the preparation of tritiated com-

0362-4803/85/090917-07$01.00

Received December 4, 1984
Revised March 22, 1985

pounds of a high specific radioactivity. A careful choice of the catalyst as well as of the solvent used for the reaction may favourably influence the reaction kinetics and reduce an undesirable ^{3}H exchange (4,5). Higher specificities of the labeling are usually obtained if using homogeneous catalysts (4). A decrease of the specific radioactivity during the reaction due to an exchange of gaseous $^{3}H_2$ with the starting material may be efficiently reduced by removal of the labile ^{1}H from the reaction vessel. This can be accomplished by using unsaturated fatty acid esters instead of free acids for the reduction.

Similar techniques were used by Glascock (5) and by Tenny et al. (6) for the synthesis of [9,10-^{3}H]stearic acid. We have earlier prepared [6,7,9,10-^{3}H]stearic acid by a catalytic tritiation of linolenic acid in dried dioxane using 5% $Pd/BaSO_4$ as catalyst (J.Filip, unpublished results).

The present paper describes the preparation of 14-methyl[2,3-^{3}H]hexadecanoic acid of a satisfactory specific radioactivity, starting from 12-methyltetradecanoic acid isolated from biological materials. The resulting product was found suitable for metabolic experiments in animals (7).

MATERIALS AND METHODS

All chemicals were of A.R. grade and solvents were redistilled before use. Methyl 12-methyltetradecanoate was isolated from the hydrolysate of wool fat by preparative gas-liquid chromatography and purified by the same method to a purity of 92%, essentially as described by Helmich and Hradec (8). Carrier-free $^{3}H_2$ was obtained from Techsnabexport, Moscow, USSR. The catalyst (5% $Pd/BaSO_4$) prepared as described by Mozingo (9) was kept over P_2O_5.

Thin-layer chromatography was performed using Silufol UV-254 plates (Kavallier, Votice, Czechoslovakia) developed with chloroform. Layers containing radioactive compounds were scanned using

a Berthold Scanner II combined with Berthold-Silena Multichannel Analyzer and HP-97S calculator. All non-radioactive compounds were analyzed by gas-liquid chromatography using a PYE Series 104 Model 24 Chromatograph equipped with dual columns and a flame ionization detector. Columns were filled with 3% SE-30 on Gas Chrom P, or with 10% diethyleneglycol succinate on Chromosorb W. A temperature gradient 120-150°C was used. Radiogas chromatography was performed on columns of 15% butanediol succinate on Chromaton N-AW, 80/100 mesh (Lachema, Brno, Czechoslovakia) at 200°C, using a Packard Model 7409 Radiogaschromatograph equipped with a flame ionization detector and a proportional counter (2 ml). Methane was used as counting gas. High-performance liquid chromatography was done using a reversed-phase system described by Helmich et al. (10). Methanol-acetone (85:15, v/v) was used for the elution. Infrared spectra were recorded on a UR 20 spectrophotometer (Zeiss, Jena, GDR). Radioactivities were counted in a Packard 2660 or Nuclear Chicago Mark II liquid scintillation spectrometer.

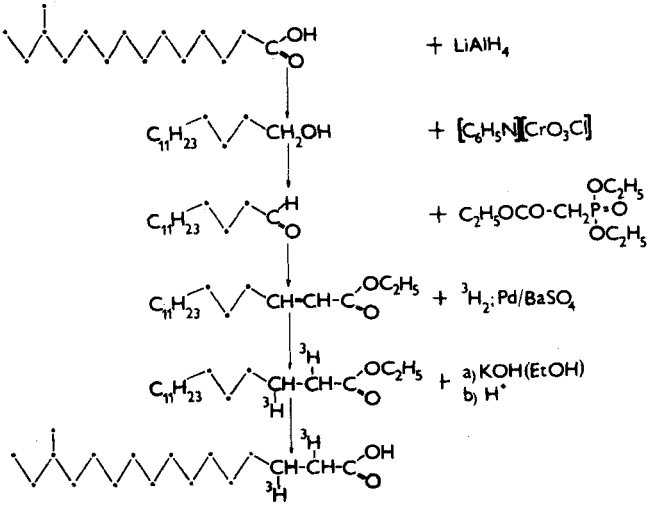

Fig.1. A scheme of reactions involved in the synthesis of 14-methyl [2,3-^{3}H]hexadecanoic acid

EXPERIMANTAL

A scheme of reactions involved in the synthesis is given in Fig.1.

12-Methyltetradecanol-1

Methyl 12-methyltetradecanoate (10 mmol, 2.56 g) was reduced by an excess of lithium aluminium hydride (1 g) in 500 ml of boiling ether for 8 hrs. Excess of the hydride was decomposed by wet ether and water and the mixture was acidified to pH 5. The ether phase was washed with 3 x 100 ml of water, dried with $MgSO_4$ and ether was evaporated. As checked by gas-liquid chromatography, the purity of the product was 85%, the yield was 2.46 g.

12-Methyltetradecanal

The primary alcohol was oxidized by pyridinium chlorochromate as described by Corey and Suggs (11). 12-Methyltetradecanol-1 dissolved in 10 ml of methylene chloride was quickly added to a suspension of 17 mmol (3.6 g) of the oxidizing mixture in 50 ml of methylene chloride and the reaction mixture was stirred for 20 min. The formation of the aldehyde was continuously checked by thin-layer chromatography. After 60 min. the dark mixture was diluted with 200 ml of dry ether, the solvent was poured-off and the dark residue was washed twice with 10 ml of ether. Pooled ether phases were passed through a column (10 g) of silicagel, the eluate was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The aldehyde prepared in this way had a purity of approx. 65% (as checked by gas-liquid chromatography), the yield was 1.95 g.

Ethyl 14-methylhexadecen-2-oate

This was prepared by the Wittig-Horner reaction of diethyl ethylphosphonoacetate using an aqueous two-phase system of NaOH-dichloromethane with tetrabutylammonium iodide as phase-transfer catalyst (12). A solution of 6.3 mmol (1.4 g) of ethyl 0,0-diethyl-α-phosphonoacetate and 6.3 mmol (1.95 g) of crude 12-methyl-

Tetradecanal in 10 ml of dichloromethane was added dropwise into a stirred two-phase system composed of 10 ml of dichloromethane-7 ml of 50% NaOH and 0.1 g of tetrabutylammonium iodide. A slightly exothermic reaction was completed in 2-3 min. The organic phase was separated, washed with water, dried over $MgSO_4$ and the solvent was evaporated. The yield was 1.1 g of a residue containing approx. 46% of the ethylester of the unsaturated acid, as revealed by thin-layer and gas-liquid chromatography. Final purification of this material by high-performance liquid chromatography gave 221 mg of the pure acid. Infrared spectrum showed bands at 1723 (C=O), 1658 (C=C) and 1184 cm^{-1}(C-O)(Fig.2).

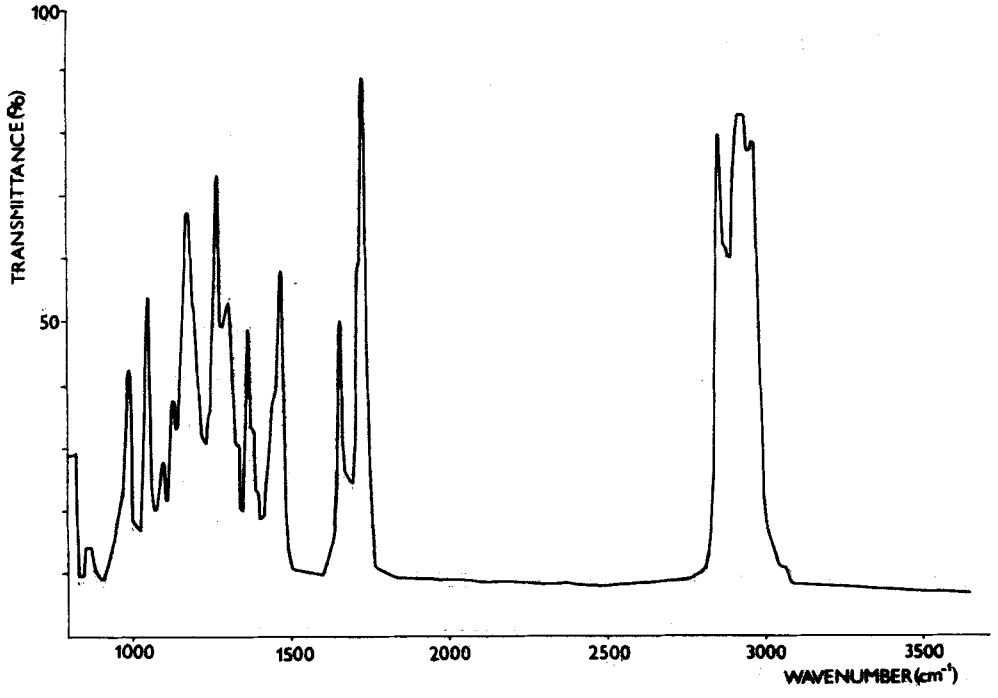

Fig.2. Infrared spectrum of ethyl 14-methylhexadecen-2-oate

Ethyl 14-methyl[2,3-^{3}H]hexadecanoate

An apparatus described by Filip et al. (13) was used for the tritiation of the ethylester of the unsaturated acid. The reaction flask was filled with 33.1 /umol (10 mg) of ethyl 14-methylhexadecen-2-oate, 6 mg of the 5% Pd/$BaSO_4$ catalyst and 0.1 ml of dried dioxane. After degassing, 5 Ci of $^3H_2$ were added by a Toepler pump and tritiation was carried on at 86 kPa and 20°C for 46 min. After that, the non-reacted $^3H_2$ was pumped-off and the reaction mixture was treated by 3x1 ml of methanol to remove labile ^3H. The yield of the tritiated product was 8.1 mg (81% of the theory), the specific radioactivity was 51.3 Ci/mmol. The radiochemical purity as determined by thin-layer chromatography was 98.4%.

14-Methyl[2,3-^{3}H]hexadecanoic acid

Ethyl 14-methyl[2,3-^{3}H]hexadecanoate (12 /umol) was dissolved in 0.5 ml of 1M NaOH in methanol and the mixture was heated at 80°C for 60 min in a sealed ampoule. After addition of HCl to pH 6 in a closed system the solvent was evaporated and 2 ml of n-hexane and 2 ml of water were added to the residue. The mixture was transferred to a liquid-liquid extractor and continuously extracted with 8 ml of n-hexane. The hexane layer was dried over anhydrous Na_2CO_3 and the solvent was evaporated. The yield was 10.57 /umol of 14-methyl[2,3-^{3}H]hexadecanoic acid (88.1% of theory) with a specific radioactivity of 51.08 Ci/mmol (87.9% of the theoretical value) and radiochemical purity was better than 97% as checked by thin-layer and high-performance liquid chromatography. The stability of ^3H in the 2,3-position was satisfactory since only 1.4% of it were released during the hydrolysis of ethyl 14-methyl[2,3-^{3}H]hexadecanoate in 1M KOH at 80°C for 60 min.

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