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A CONVENIENT SYNTHESIS OF 8- AND 12-CARBON DEUTERIUM-LABELLED ALDEHYDIC ESTERS FOR USE IN THE PREPARATION OF LABELLED FATTY ACIDS

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SUMMARY

Methyl 8-oxooctanoate-4,5-d_2 and methyl 12-oxododecanoate-4,5,8,9-d_4 were prepared for use as intermediates in the synthesis of a series of labelled fatty acid methyl esters. Synthesis of the two compounds began with monoozonization and sodium acetate cleavage of 1,4-cyclooctadiene and 1,5,9-cyclododecatriene, respectively. The resultant unsaturated aldehydic acids were converted to the acetal esters. The acetal esters were deuterated with Wilkinson's catalyst and hydrolyzed to the deuterium-labelled aldehydic esters. Overall yields were 47% and 49% and isotopic purities 97% and 89%, respectively.

Key Words: Ozonolysis, deuterium, aldehydic ester.

Our studies on the metabolism of configurational and positional fatty acid isomers in humans require the preparation of multi-gram quantities of deuterium-labelled fats. High yield syntheses were developed by utilizing a variation of Bergelson's procedure (1) in which the final double bond is incorporated by the Wittig coupling of a deuterium-labelled, alkenyl triphenylphosphonium salt to the appropriate aldehydic ester (2). These syntheses, however, result in placement of the deuterium atoms at the alkyl end of the fatty acid molecule and cannot easily be applied to the preparation

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of ω-3 fatty acids (i.e. 12,15-18:2). The synthesis of deuterium-labelled ω-3 fatty acids by this procedure would require the purchase of expensive deuterium-labelled fragments or incorporation of the deuterium atoms on the double bonds. The latter procedure could result in the generation of isotope effects.

We have prepared methyl 8-oxooctanoate-4,5-d_2 and methyl 12-oxododecanoate-4,5,8,9-d_4 with isotopic purities of 97 and 89%, respectively. These compounds were prepared by the monoozonization (3) of 1,5 cyclooctadiene and 1,5,9 cyclododecatriene to yield the mono- or diunsaturated aldehydic acids, which were converted to the corresponding acetal esters by HCl/CH_3OH (4). The unsaturated acetal esters were reduced to saturated acetal esters by use of Wilkinson's catalyst [$(Ph_3P)_3RhCl$] and deuterium gas (5). The deuterium-labelled acetal esters were hydrolyzed to the aldehydic esters by $H_2O/CH_3CN/HCl$ when required (4). Overall yields were 47% for the 8-carbon aldehydic ester-d_2 and 49% for the 12-carbon aldehydic ester-d_4. Attempts to prepare the 6-carbon aldehydic ester-d_2 by monoozonization of 1,4 cyclohexadiene were not successful.

The unsaturated aldehydic esters cannot be reduced by Wilkinson's catalyst. Catalytic activity ceased due to abstraction, by the catalyst, of carbon monoxide from the substrate and formation of a yellow precipitate [trans-$RhCl(CO)(PPh_3)_3$] (6). Reduction of the unsaturated acetal esters proceeded smoothly. Unlike the aldehydic ester, the acetal ester does not trimerize (4) and can be stored for years before hydrolysis and use. Isotopic purities are also higher than in other procedures for preparation of labelled aldehydic esters [i.e., D_2O/pyridine (7)].

Purities of the deuterium-labelled aldehydic esters need not be >99%. As we have indicated (8), a combination of silver resin and C18 reverse phase chromatography can be used to remove a wide variety of impurities from the prepared deuterium-labelled fatty acid methyl esters.

EXPERIMENTAL

Reaction products were analyzed on a Packard 428 Gas Chromatograph (GC) equipped with a 30.0 X 1.0 cm 3% EGSS-X column. A flame ionization detector and helium carrier gas were utilized. Isotopic purities and deuterium

distributions (9) were measured on a Finnigan 4500 Mass Spectrograph (MS) in chemical ionization mode (conditions: isobutane reagent gas, 70 eV), which was interfaced with a 30 m X 0.32 mm Supelcowax 10 capillary column. The column was programmed from 100 to 265°C at 5°C/min. Helium was used as carrier gas. Ozonolyses were carried out with a Welsbach T816 Ozonator (12 g ozone/h; oxygen feed).

Methyl-8-oxooctanoate-4,5-d_4 (See Figure 1 for preparation of the 12-carbon analogue). 1,5 cyclooctadiene (48 g; .44 mol) was monoozononized by the method of Siclari et al. (3). Fat Red B indicator was used to determine when the reaction was complete (10). Cleavage of the ozonid intermediate by sodium acetate and work-up afforded 42.8 g of 8-oxo-4-octenoic acid (96% pure, 60% yield, bp 140-142°C/0.20 mm Hg). The acid was esterified by reaction with CH_3OH/ trimethylorthoformate/HCl (4) to produce the unsaturated acetal ester, methyl 8,8-dimethoxy-4-octenoate (39.5 g; 95% pure; 87% yield; bp 80-82°C/0.10 mm Hg). The unsaturated acetal-ester (39.0 g) was reduced with Wilkinson's catalyst and deuterium gas in benzene (5) to yield methyl 8,8-dimethoxyoctanoate-4,5-d_2 (36.8 g; 95% pure; 94% yield). Hydrolysis of the deuterium-labelled acetal ester (20.0 g) with H_2O/CH_3CN/HCl (4) resulted in 14.2 g of methyl 8-oxooctanoate-4,5-d_4 (97% pure; 95% yield, bp 91-94°C/0.2 mm Hg) having a deuterium distribution of 1.6% d_1, 97.2% d_2 and 1.1% d_3.

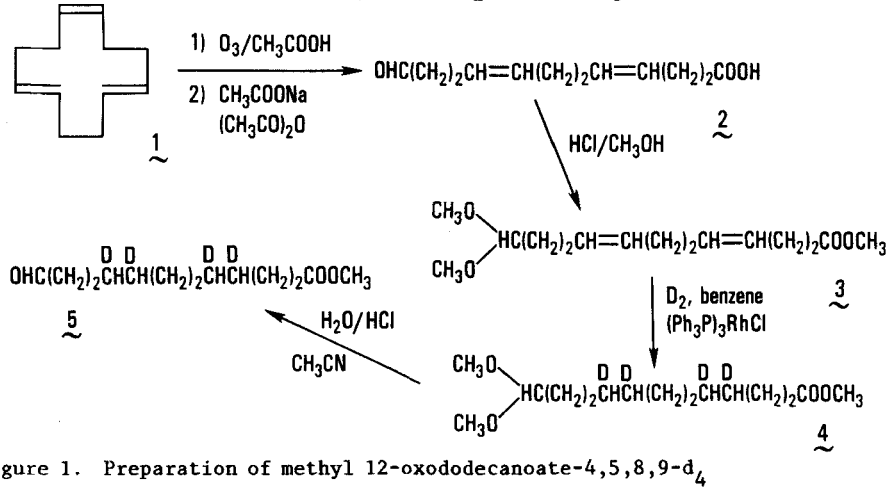

Figure 1. Preparation of methyl 12-oxododecanoate-4,5,8,9-d_4

Methyl 12-oxododecanoate-4,5,8,9-d_4 (See Figure 1). 1,5,9 cyclododecatriene, 1 (35.6 g; 0.22 mol) was ozonized and, without further purification, the unsaturated aldehydic acid 2 was esterified by use of CH_3OH/trimethylorthoformate/HCl to compound 3 (42,3 g; 85% pure; 60% yield; bp 122-130°C/0.10 mm Hg). Compound 3 (15.0 g; 4.6 X 10^{-2} mol) was reduced via Wilkinson's catalyst and deuterium gas to compound 4 (13.0 g; 78% pure; 78% yield; bp 106-110°C/0.05 mm Hg) and chromatographed in 3 batches on a 5 X 25 cm (5 μ particle size) C18 reverse phase chromatography column (Serva Feinbiochemica, Heidelberg, Germany) by utilizing CH_3CN as solvent to yield 11.0 g of 4 (92% pure; 99% recovery). Hydrolysis with H_2O/CH_3CN/HCl resulted in compound 5 (8.7 g; 92% pure; 96% yield, bp 76-78°C/0.2 mm Hg). Compound 5 was determined to have a deuterium distribution of 0.2% d_2, 6.4% d_3, 89.4% d_4, 3.8% d_5 and 0.1% d_6.

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