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Research Article

Stereocontrolled synthesis of (7E,9Z)-[9,10-^{2}H]-conjugated linoleic acid

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Summary

To study the metabolism of minor conjugated linoleic acid isomers (CLAs), (7E,9Z)-[9,10-^{2}H] CLA was prepared in three steps from a conjugated enyne precursor. In the labelling step, deuterium atoms were introduced by partial reduction of the triple bond using deuterated disiamylborane and acetic acid-d_4. Deuterated (7E,9Z) CLA was obtained with high isotopic enrichment (> 99%) and purity greater than 95%. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: conjugated linoleic acid (CLA); deuterium; octadecadienoic acid; sodium borodeuteride; stereocontrolled

Introduction

Conjugated linoleic acid (CLA) is a collective term which refers to positional and geometrical isomers of linoleic acid with conjugated double bonds. CLA isomers are present in edible fats derived from milk[1] and ruminant meat.[2] Their metabolic pathway and biological effects, such as anticarcinogenic activity and ability to reduce body fat, have been extensively studied over the past two decades.[3,4] Most of the studies on CLAs have been conducted with (9Z,11E) and/or (10E,12Z) CLAs which are the two predominant isomers. Consequently, the metabolism and the effects of minor CLAs remain partly unclear. In order to investigate further the metabolic fate of minor CLAs, we have prepared (7E,9Z)-[9,10-^{2}H]-octadeca-7,9-dienoic acid **4** ((7E,9Z)-[9,10-^{2}H] CLA), a deuterated analogue of a minor isomer recently identified by Yurawecz *et al.*[5]

In the literature, many papers have described the preparation of deuterated skipped polyunsaturated fatty acids using alkynes, Lindlar catalyst and

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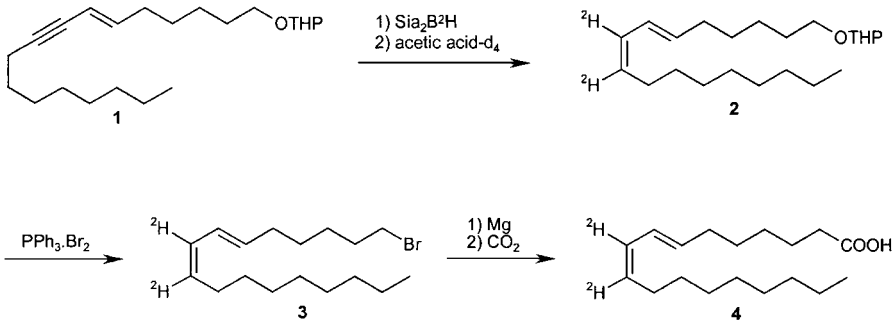
deuterium gas.[6] Unfortunately, using this methodology, partial reduction of conjugated enynes often provides conjugated polyalkenes contaminated by over-reduced compounds and/or geometrical isomers.[7,8] Moreover, as reported by Adlof for the preparation of methyl (9Z,11E)-[9,10-^{2}H]-octadecadienoate ((9Z,11E) CLA),[8] the isotopic purity of deuterated conjugated dienes can be lower than the value usually associated with this reaction.

Recently, Svătos *et al.*[9] synthesised deuterated linolenic acid from a methylene interrupted polyacetylenic precursor using bis (2-deuteriocyclohexyl)borane-B-^{2}H and deuterated acetic acid. The labelled fatty acid was obtained with high geometrical and isotopic purities. This result prompted us to investigate the preparation of deuterated CLA. In this report, we describe the stereocontrolled synthesis of (7E,9Z)-[9,10-^{2}H] CLA **4** using deuterated disiamylborane and acetic acid-d_4 as labelling reagents.

Results and discussion

The synthesis of 2-((E)-heptadec-6-en-8-ynyloxy)-tetrahydro-pyran **1** was carried out using the procedure previously developed in our laboratory for the preparation of (9Z,11E)- and (10E,12Z)-[1-^{14}C] CLA isomers.[10] This multi-step synthesis involved the sequential substitution of (E)-1,2-dichloro-ethene. A first metal-catalysed cross-coupling reaction between (E)-1,2-dichloro-ethene and 1-decyne with $Pd(PPh_3)_4$ furnished a conjugated chloroenyne.[11] This was coupled, in the presence of $Fe(acac)_3$,[12] with the Grignard reagent derived from 2-(5-chloro-pentyloxy)-tetrahydro-pyran. The conjugated enyne **1** was obtained as key intermediate for the labelling (51% overall yield from 1-decyne). Compound **1** (1 eq.) was submitted to partial reduction with deuterated disiamylborane (prepared in situ from sodium borodeuteride (2 eq.), 2-methyl-but-2-ene (4 eq.) and $BF_3.Et_2O$ (1.56 eq.)). After protonolysis with deuterated acetic acid, (6E,8Z)-[8,9-^{2}H]-heptadeca-6,8-dienyloxy-tetrahydro-pyran **2** was obtained in 68% yield. At this stage, no geometric isomers of conjugated diene **3** were detected by ^{1}H and ^{13}C NMR. Deuterated heptadecadienol was also produced as side-product ($<5\%$ yield). Bromination of **2** with triphenyl-phosphine dibromide[13,14] gave (6E,8Z)-[8,9-^{2}H]-1-bromo-heptadeca-6,8-diene **3** (82% yield). Formation of the heptadecadienylmagnesium bromide and reaction with carbon dioxide furnished (7E,9Z)-[9,10-^{2}H]-octadeca-7,9-dienoic acid **4** in 50% yield from **3** (Scheme 1). Isotopic enrichment ($>99\%$) was determined by mass spectrometry (ESI/TOF). The chemical purity of the acid **4** was found to be 95.5% by RP HPLC (column Zorbax SB C18). Compound **4** was submitted to esterification using a 14% BF_3 solution in methanol.[15] The resulting CLA methyl ester was analysed by silver-ion HPLC[16] (column Chromspher 5 Lipids) and its purity was



Scheme 1. Synthesis of (7E,9Z)-[9,10-^{2}H]-octadeca-7,9-dienoic acid

97.8%. Moreover, only traces of two geometric isomers (respectively, 0.8 and 4%) were found to be present by GC/MS analysis of the methyl ester (column HP-5MS).

In conclusion, we have synthesised deuterated (7E,9Z) CLA isomer **4** with high chemical and geometric purities (> 95%). Compound **4** was free of over-reduced side-products and its isotopic enrichment was greater than those previously reported for the preparation of deuterated CLA using Lindlar catalyst. More generally, this methodology can be a convenient alternative for the preparation of deuterated analogues of natural products with a conjugated diene moiety.

Experimental

Chemical reagents and solvents were from Aldrich, Fluka or SDS. Solvents were purified before use: diethyl ether and tetrahydrofuran were distilled from sodium/benzophenone ketyl; dichloromethane was distilled from calcium hydride. All purified solvents were stored over molecular sieves. Unless otherwise stated, reactions were carried out under argon.

Flash chromatography was performed using silica gel 60 (0.040–0.063 mm) from Merck. Analytical TLC was performed with silica gel 60F254 (Merck) and visualization was carried out with an anisaldehyde solution (anisaldehyde 12.5 ml; acetic acid 5 ml; sulphuric acid 17 ml; 95% ethanol 450 ml). NMR spectra were recorded on a Bruker AC 300 spectrometer (7.05 T; 300.13 MHz (^{1}H); 75.47 MHz (^{13}C)) and the chemical shifts were reported in ppm. Isotopic enrichment was determined by mass spectrometry (Mariner Biospectrometry Workstation (ESI/TOF)). HPLC analyses were performed with a Shimadzu LC-10AS pump fitted with a Shimadzu SCL-10A system controller. The UV detector was an LDC 3200 analytical spectro monitor. GC/MS analyses were effected on an HP 6890 Series gas chromatograph system coupled to an HP5973 mass selective detector.

2-((E)-heptadec-6-en-8-ynyloxy)-tetrahydro-pyran **1**

Conjugated enyne **1** was prepared from 1-decyne, (E)-1,2-dichloro-ethene and the Grignard reagent derived from 2-(5-chloro-pentyloxy)-tetrahydro-pyran using the procedure described for the synthesis of [1-^{14}C]-(9Z,11E) and (10E,12Z) CLA isomers[10] (overall yield from 1-decyne: 51%). TLC: Rf = 0.35 (pentane/diethyl ether (96/4)). ^{1}H NMR ($CDCl_3$): 0.85 (t; $J = 6.7$ Hz; 3H), 1.1–1.9 (m; 24H), 2.05 (td; $J = 6.7$, 6.7 Hz; 2H), 2.25 (t; $J = 6.7$ Hz; 2H), 3.35 (dt; $J = 9.2$, 6.7 Hz; 1H), 3.5 (m; 1H), 3.7 (dt; $J = 9.2$, 6.7 Hz; 1H), 3.85 (m; 1H), 4.55 (m; 1H), 5.4 (d; $J = 15.9$ Hz; 1H), 6 (dt; $J = 15.9$, 6.7 Hz; 1H). ^{13}C NMR ($CDCl_3$): 13.82, 19.09, 19.41, 22.39, 25.27, 25.46, 28.44, 28.6, 28.67, 28.86, 28.92, 29.28, 30.51, 31.58, 32.61, 62.05, 67.19, 78.87, 88.51, 98.57, 109.77, 142.7.

2-((6E,8Z)-[8,9-^{2}H]-heptadeca-6,8-dienyloxy)-tetrahydro-pyran **2**

A suspension of a 2 M solution of 2-methyl-but-2-ene in THF (19.2 ml; 38.4 mmol; 4 eq.) and sodium borodeuteride (795 mg; 19 mmol; 1.98 eq.)) was treated, at 0°C, with $BF_3.Et_2O$ (1.9 ml; 15 mmol; 1.57 eq). The resulting mixture was stirred for 2.25 h at room temperature and then cooled to 0°C. Compound **1** (3.2 g; 9.58 mmol; 1 eq) in 8 ml of anhydrous THF was added dropwise. The reaction mixture was allowed to warm to 20°C over 4 h and then cooled to 0°C. Acetic acid-d_4 (4 ml) was added and the mixture was stirred overnight at room temperature. The mixture was treated, at 0°C, with a 7.5 N NaOH solution until the pH was alkaline and then 7 ml of a 30% H_2O_2 solution were added. After stirring for 1 h at room temperature, 50 ml of water and 100 ml of diethyl ether were added. The aqueous layer was extracted with 100 ml of diethyl ether. The combined organic layers were washed with water, dried over Na_2SO_4, filtered and concentrated. The residue was chromatographed on silica gel with pentane/diethyl ether (97/3) giving 2-((6E,8Z)-[8,9-^{2}H]-heptadeca-6,8-dienyloxy)-tetrahydro-pyran **2** (2.2 g; 6.51 mmol; 68% yield). TLC: Rf = 0.35 (pentane/diethyl ether (96/4)). ^{1}H NMR ($CDCl_3$): 0.85 (t; $J = 6.7$ Hz; 3H), 1.1–1.9 (m; 24H), 2.08 (m; 4H), 3.33 (dt; $J = 9.2$, 6.7 Hz; 1H), 3.45 (m; 1H), 3.7 (dt; $J = 9.2$, 6.7 Hz; 1H), 3.85 (m; 1H), 4.54 (m; 1H), 5.6 (dt; $J = 15.3$, 6.7 Hz; 1H), 6.25 (d; $J = 15.3$ Hz; 1H). ^{13}C NMR ($CDCl_3$): 13.82, 19.41, 22.29, 22.42, 25.27, 25.59, 27.31, 29.02, 29.25, 29.34, 29.47, 30.51, 31.64, 32.55, 61.95, 67.23, 98.51, 125.49, 133.93.

Impure alcohol (120 mg; yield: $<5\%$) were also recovered after flash chromatography on silica gel (pentane/diethyl ether (50/50)).

(6E,8Z)-[8,9-^{2}H]-1-bromo-heptadeca-6,8-diene **3**

Compound **2** (2.2 g; 6.51 mmol) was dissolved in 5 ml of CH_2Cl_2. The resulting solution was added dropwise at 0°C to a slurry of triphenylphosphine

dibromide (6.2 g; 14.69 mmol) in 20 ml of CH_2Cl_2. After the addition, the reaction mixture was stirred at room temperature for 1.5 h, washed with a saturated solution of sodium bicarbonate, then with water, dried over Na_2SO_4 and filtered. Dichloromethane was partially removed under vacuum and pentane was added to precipitate triphenylphosphine oxide. The residue was purified by chromatography on silicagel with pentane giving (6E,8Z)-[8,9-^{2}H]-1-bromo-heptadeca-6,8-diene **3** (1.7 g; 5.36 mmol; 82% yield). TLC: Rf = 0.7 (pentane). ^{1}H NMR ($CDCl_3$): 0.88 (t; $J = 6.7$ Hz; 3H), 1.2–1.5 (m; 16H), 1.86 (quint; $J = 6.7$ Hz; 2H), 2.05–2.2 (m; 4H), 3.4 (t; $J = 6.7$ Hz; 2H), 5.62 (dt; $J = 15.3$, 7 Hz; 1H), 6.3 (d; $J = 15.3$ Hz; 1H). ^{13}C NMR ($CDCl_3$): 13.91, 22.49, 27.37, 27.53, 28.31, 29.09, 29.31, 29.51, 31.71, 32.42, 32.48, 33.49, 125.78, 133.51.

(7E,9Z)-[9,10-^{2}H]-octadeca-7,9-dienoic acid **4**

Bromide **3** (1.15 g; 3.14 mmol) dissolved in 5 ml of anhydrous diethyl ether was added to magnesium turnings (430 mg; 17.7 mmol) and a crystal of iodine in 3 ml of anhydrous diethyl ether. The reaction mixture was heated to reflux for 1 h. The resulting solution of Grignard reagent was carbonated at -20°C with CO_2. After stirring at -20°C for 2 h, 10 ml of 5% NH_4Cl aqueous solution were added. The mixture was diluted with 100 ml of diethyl ether and 100 ml of water. A 1 N H_2SO_4 solution was added dropwise until the aqueous layer was acidified to pH 3. After decantation, the ethereal layer was washed with water, dried over Na_2SO_4, filtered and concentrated. Chromatography on silica gel with pentane/diethyl ether/acetic acid (80/20/0.1) gave (7E,9Z)-[9,10-^{2}H]-octadeca-7,9-dienoic acid **4** (440 mg; 1.56 mmol; 50% yield). TLC: Rf = 0.35 (pentane/diethyl ether/acetic acid (80/20/0.1)). ^{1}H NMR ($CDCl_3$): 0.88 (t; $J = 6.7$ Hz; 3H), 1.2–1.5 (m; 18H), 1.64 (quint; $J = 7.3$ Hz; 2H), 2–2.2 (m; 4H), 2.34 (t; $J = 7.3$ Hz; 2H), 5.62 (dt; $J = 15.3$, 6.7 Hz; 1H), 6.3 (d; $J = 15.3$ Hz; 1H). ^{13}C NMR ($CDCl_3$): 13.85, 22.45, 24.3, 27.34, 28.37, 28.79, 29.09, 29.28, 29.51, 31.67, 32.39, 33.81, 125.65, 133.71, 180.19. Isotopic enrichment: > 99% (mass spectrometry (ESI/TOF)). RP-HPLC: Column Zorbax SB C18 (250 × 4.6 mm). Solvent system: ethanol/water/trifluoroacetic acid (70/30/0.1). Flow rate: 1 ml/min. Detection: UV 230 nm. Chemical purity: (7E,9Z)-[9,10-^{2}H]-octadeca-7,9-dienoic acid **4** (r.t. = 21.9 min; 95.5%), impurity (r.t. = 25.6 min; 4.5%). Silver-ion HPLC: Column Chromspher 5 Lipids (250 × 4.6 mm). Solvent system: hexane/acetonitrile (1000/1). Flow rate: 1 ml/min. Detection: UV 230 nm. impurity (r.t. = 18 min; 2.2%), (7E,9Z)-[9,10-^{2}H]-octadeca-7,9-dienoic acid, methyl ester (r.t. = 29.7 min; 97.8%). GC/MS: Column HP-5MS (30 m × 0.25 mm × 0.25 μm). Column temperature: 60 (4 min)–300°C (5 min); 18°C/min. Carrier gas: He (0.8 ml/min). Injector temperature: 250°C. (7E,9Z)-[9,10-^{2}H]-octadeca-7,9-dienoic acid, methyl ester

(r.t. = 15.47 min; 95.2%; $m/z = 296\,M^+$), geometric isomers (r.t. = 15.61 min; 0.8%; $m/z = 296\,M^+$) and (r.t. = 15.69 min; 4%; $m/z = 296\,M^+$).

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