SYNTHESIS OF 4-HYDROXY[4-^{3}H]-2(E)-NONEN-1-AL-DIETHYLACETAL

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SUMMARY

4-Hydroxy-2(E)-nonen-1-al-diethylacetal **3** (HNE-DEA) was prepared by condensation of the Grignard compound derived from propiolaldehyde diethylacetal and n-hexanal followed by reduction of the resulting 4-hydroxy-2-nonyn-1-al-diethylacetal **2** with $LiAlH_4$ according to the literature procedure with minor modifications. Swern oxidation [DMSO + $(COCl_2)$] of **3** gave 30% yield of 4-oxo-2(E)-nonen-1-al-diethylacetal **5**. [^{3}H]$NaBH_4$ and [^{2}H]$NaBH_4$ reduction of **5** gave rise respectively to [4-^{3}H]HNE-DEA (specific activity 222 GBq/mmol) and [4-^{2}H]HNE-DEA.

KEY WORDS: Lipid peroxidation product: 4-Hydroxy-2-nonenal, 4-Hydroxy-2(E)-nonen-1-al-diethylacetal, 4-Oxo-2(E)-nonen-1-al-diethylacetal, 4-Hydroxy[4-^{3}H]-2(E)-nonen-1-al-diethylacetal, 4-Hydroxy[4-^{2}H]-2(E)-nonen-1-al-diethylacetal.

INTRODUCTION

The free radicals generated in biological systems play an important role in causing oxidative damage to living cells, particularly to the biomembranes. Among many compounds, 4-hydroxy α,β-unsaturated aldehydes (hydroxy alkenals) are formed as degradation

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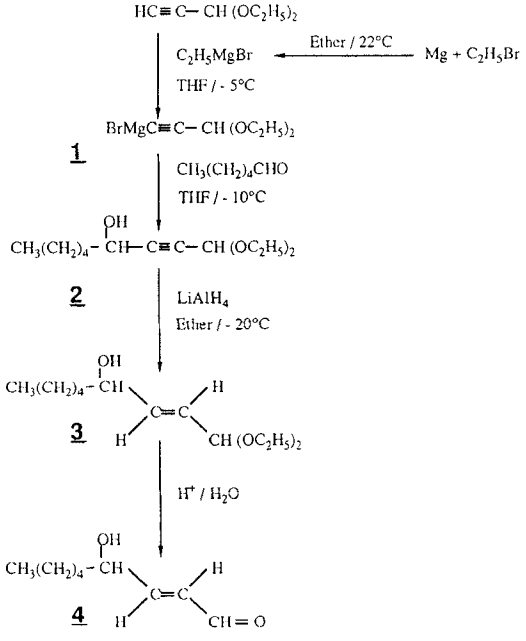
products of membranes during lipid peroxidation. One of the major released hydroxy alkenal is 4-hydroxy-2-nonenal (1). It has been shown that 4-hydroxy-2-nonenal is a strong alkylating agent and plays a major part to induce genotoxic (2,3), cytotoxic (2) and mutagenic effects (4).

In spite of many studies published on 4-hydroxy-2-nonenal in the past years, there are very few reports of the biotransformation of this compound, especially in vivo. Hence, a method was designed and developed to synthesize [4-^{3}H]HNE-DEA. The synthetic method consists of preparing the corresponding 4-oxo compound from HNE-DEA **3**, which was then reduced with [^{2}H] or [^{3}H] sodium borohydride, to give the corresponding isotopically labelled compounds.

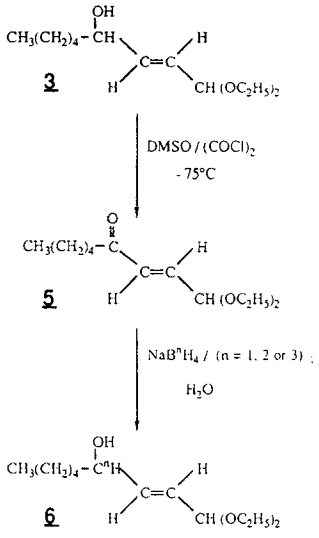
RESULTS AND DISCUSSION

HNE-DEA **3** was prepared from propiolaldehyde diethylacetal and 1-hexanal (scheme 1) according to a method described by Esterbauer and Weger (1) with minor modifications.

Then, the selective oxidation of the secondary alcohol function of HNE-DEA **3** was studied with various oxidizing agents. Thus pyridinium chlorochromate (5), aluminium



Scheme 1: Synthesis of 4-hydroxy-2(E)-nonen-1-al-diethylacetal **3**, and 4-hydroxy-2(E)-nonen-1-al **4**.



Scheme 2: Synthesis of 4-oxo-2(E)-nonen-1-al-diethylacetal **5**, and 4-hydroxy (4^nH)-2(E)-nonen-1-al-diethylacetal **6**.

tert-butoxide (6), activated manganese dioxide (7), activated dimethysulphoxide (DMSO) (8) (Swern oxidation) were tried. The latter two reagents gave the expected 4-oxo product. The Swern oxidation with oxalyl chloride activated DMSO was selected because the end product was cleaner (Scheme 2). The yield of the purified product was around 25-30 %. No attempts were made to optimize it. The expected structure was confirmed in all respects by various analytical methods. The 4-oxo compound was then reduced with $NaBH_4$ to have HNE-DEA **6**, or with [^{3}H]$NaBH_4$ or [^{2}H]$NaBH_4$ to give its tritiated or deuterated homologue. The radio-labelled product was compared with the protonated and deuterated homologues by thin layer chromatography and by high performance liquid chromatography (HPLC), after acid hydrolysis of the diethylacetal. The radiochemical purity of 4-hydroxy[4-^{3}H]-2(E)-nonenal was 95 %, as determined by HPLC and its specific activity was very high: 222 GBq/mmol. The determination of urinary metabolites of 4-hydroxy[4-^{3}H]-2(E)-nonenal in the case of rats is under study. Part of the results will appear soon (9).

EXPERIMENTAL

The chemicals used in the syntheses were purchased from Sigma-Aldrich Chimie, L'Isle D'Abeau Chesnes, B.P. 701, 38297 St Quentin Fallavier Cedex, France.

Thin layer chromatography (TLC) was performed on silica gel 60F 254 (Merck) plates (0.25 mm thickness). For column chromatography 0.063-0.200 mm particle size silica gel (Merck) was used.

The HPLC system consisted of two 420 Kontron pumps with a gradient former 491 and a Spectra Physics UV 150 detector (set at 223 nm). Radioactivity detection was carried out with a radiomatic Flo-one-A-200 instrument (Radiomatic, La-Queue-Lez-Yvelines, France) with Flo-scint II as scintillation cocktail (Packard Instrument Co., Downers Grove,Il, USA.).

The elemental microanalyses were performed on a Carlo Erba 1106 analyser at the Ecole Nationale Supérieure de Chimie, Toulouse, France. The infrared spectra were recorded on a Perkin-Elmer Spectrophotometer FT model 1600. The ultraviolet spectra were obtained on a Kontron Spectrophotometer Uvikon model 810. The nuclear magnetic resonance (NMR) spectra were recorded at the Laboratoire de Chimie de Coordination, CNRS, Toulouse, France, on a Bruker AMX 400 spectrometer. The compounds were in deuterated chloroform ($CDCl_3$) solutions at room temperature. The chemical shifts (δ/ppm) are referred to tetramethylsilane (δ_{TMS}=0.0 ppm).

The mass spectra were obtained on a Nermag R-10-10H (Delsi Nermag Inst., Argenteuil, France) single quadrupole mass spectrometer working in the negative ion mode. FAB experiments were achieved with an M-Scan FAB gun (M-Scan Ltd., Ascot, UK) and Xenon gas was used for bombardment at an accelerating voltage of 8kV, with 1-2mA as discharge current. Samples were prepared by mixing 1µL of the sample solution (1µg/µL in methanol) with the matrix thioglycerol.

4-Hydroxy-2(E)-nonen-1-al-diethylacetal (HNE-DEA) 3

HNE-DEA was prepared following scheme 1 according to (1) with minor modifications. The Grignard compound derived from propiolaldehyde diethylacetal **1**, was condensed with hexanal to form 4-hydroxy-2-nonyn-1-al-diethylacetal **2**. The CC triple bond was then reduced with $LiAlH_4$ to the trans CC double bond giving rise to HNE-DEA **3**.

^{1}H-NMR δ: 4.90 (dd, J=5.1, 1.1 Hz, 1H, H-1), 5.87 (ddd, J=15.7, 6.2, 0.9 Hz, 1H, H-1), 5.70 (ddd, J=15.7, 5.1, 1.1 Hz, 1H, H-3), 4.16 (ddd, J=6.1, 1.1, 5.1 Hz, 1H, H-4), 1.56 (m, 4H, H-5, 6), 1.30 (m, 4H, H-7, 8), 0.89 (t, J=8.0 Hz, 3H, H-9), 5.31 (d, J=1.1

Hz, H, OH), 3.66 (m, 2H, CH_2 (OEt) a,a') 3.53 (m, 2H, CH_2 (OEt) b,b'), 1.23 (t, J=8.0 Hz, 3H, CH_3 (Et)). FAB-MS: m/z 253 $(M+Na)^+$ and 269 $(M+K)^+$

4-Oxo-2(E)-nonen-1-al-diethylacetal 5

Under nitrogen a solution of oxalyl chloride (0.5 mL, 0.55 mmol) in dichloromethane (15 mL) was placed in a 50 mL three-necked round-bottomed flask, equipped with an efficient stirrer, and two dropping funnels containing DMSO (0.85 mL, 11 mmol) diluted with dichloromethane (3 mL), and HNE-DEA (1.15 g, 5 mmol) also in dichloromethane (5 mL), respectively. The solution of DMSO was added to the stirred solution of oxalyl chloride at -70°C to -75°C. The reaction mixture was stirred for 5 minutes and then the solution of HNE-DEA was added within 5 minutes. Triethylamine (3.5 mL, 50 mmol) was added and the reaction mixture was stirred for 5 minutes more and then allowed to warm to room temperature. Then water (25 mL) was added and the organic layer was separated. The aqueous layer was reextracted with dichloromethane (50 mL). The organic layers were combined, washed with saturated sodium chloride solution (50 mL), dried over anhydrous magnesium sulphate, filtered and the solvent was removed under reduced pressure, leaving 1g of oxidized product. Thin layer chromatography on silica gel 60F 254, benzene-ethylacetate (95:5), as the solvent system separated the two major reaction products. The product with Rf=0.75 was the expected 4-oxo compound **5** in 25% yield and the secondary product with Rf=0.70 was in 36% yield (10). Analytical data for **5**:

^{1}H-NMR δ: 5.03 (dd, J=4.3 Hz, 1H, H-1), 6.61 (dd, J=16.2, 4.3 Hz, 1H, H-2), 6.32 (dd, J=16.2, 1.3 Hz, 1H, H-3), 2.55 (t, J=7.5 Hz, 2H, H-5), 1.59 (tt, J=7.4, 7.4 Hz, 2H, H-6), 1.28-1.31 (m, 4H, H-7, 8), 0.86 (t, J=7.0 Hz, 3H, H-9), 3.63 (dq, J=14.1,7.1 Hz, 2H, CH_2 (OEt)).

^{13}C-NMR δ: 13.83 (q, J_{CH}= 125 Hz, C-9), 15.11 (q, J_{CH}=125 Hz, CH_3 (Et)), 22.37 (t, J=125 Hz, C-8), 23.60 (t, J=125 Hz, C-7), 31.32 (t, J=125 Hz, C-6), 40.30 (t, J=125 Hz, C-5), 61.41 (t, J=130 Hz, OCH_2 (Et)), 99.56 (d, J=158 Hz, C-1), 131.54 (d, J=158 Hz, C-3), 140.81 (d, J=158 Hz, C-2), 200.63 (s, C-4).

UV. λmax-nm (ε): (Ethanol) 214 (10217); IR. cm^{-1}: (neat) 1701.6, 1681.5 ($\nu_{C=O}$); 1642.9 ($\nu_{C=C}$); 1054.8-1136.6 (ν_{OC2H5}).

Anal. calcd. for $C_{13}H_{24}O_3$: C, 68.42; H, 10.52; O, 21.05; Found: C, 68.41; H, 10.80; O,21.03. FAB-MS: m/z 251 $(M+Na)^+$ and 267 $(M+K)^+$

4-Hydroxy[4-^{2}H]-2(E)-nonen-1-al-diethylacetal 6

4-Oxo-2(E)-nonen-1-al-diethylacetal (12 mg, 0.053 mmol) was dissolved in dry methanol (4 mL). At room temperature (22°C), sodium borodeuteride (20 mg, 0.47 mmol) was added during 10 minutes, in three equal portions. The solvent was removed at reduced pressure. The deuterated product was extracted in ether (30 mL) and washed with cold water till the pH of the washings was neutral. The organic phase was dried over anhydrous magnesium sulphate, filtered and solvent was removed in vacuo. The reduction is almost quantitative.

^{1}H-NMR δ: 4.86 (d, J=4.0 Hz, 1H, H-1), 5.89 (dd, J=15.6, 4.0 Hz, H-2), 5.67 (dt, J=15.6, 6.5 Hz, 1H, H-3), 1.59 (m, 4H, H-5, 6), 1.30 (m, 4H, H-7, 8), 0.89 (broad t, 3H, H-9), 3.65 (m, 2H, CH_2 (OEt) a, a'), 3.51 (m, 2H, CH_2 (OEt) b,b'), 1.23 (t, J=8.0 Hz, 3H, CH_3 (Et)). FAB-MS: m/z 254 $(M+Na)^+$ and 270 $(M+K)^+$

4-Hydroxy[4-^{3}H]-2(E)-nonen-1-al-diethylacetal 6

The tritiated product was prepared as described above for [4-^{2}H]HNE-DEA 6 using [^{3}H]$NaBH_4$. This radiosynthesis was carried out at CEA, Service des Molécules Marquées, CEN, Saclay, France. The radiochemical purity was determined by HPLC (after acid hydrolysis) and was found to be 95%, while its specific activity was 222 GBq/mmol. The chromatographic properties (e.g. HPLC, TLC) of the tritiated HNE-DEA were compared with the authentic sample donated by Professor H. Esterbauer.

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(10) According to its NMR spectra, the secondary oxidized product has the following structure: $CH_3(CH_2)_4\,CO\,CH{=}CH\,CH_2\,O\,CH_2CH_3$ (4-oxo-1-ethoxy-2(E)-nonen): ^{1}H-NMR ($CDCl_3$) δ: 4.11 (dd, J=4.4,2.0 Hz, 1H, H-1), 6.78 (dt, J=16.0,4.4 Hz, 1H, H-2), 6.29 (dt, J=16.0,2.0 Hz, 1H, H-3), 2.51 (t, J=7.6 Hz, 2H, H-5), 1.57 (tt, J=8.0,8.0 Hz, 2H, H-6), 1.27 (m, 4H, H-8), 0.85 (t, J=7.1 Hz, 3H, H-9), 3.50 (q, J=7.0 Hz, 2H, CH_2 (OEt)), 1.20 (t,J=7.0 Hz, 3H, CH_3 (OEt)).

^{13}C-NMR ($CDCl_3$) δ: 13.80 (q, J=125 Hz, C-9), 15.03 (q, J=125 Hz, CH_3 (Et)), 22.35 (t, J=125 Hz, C-8), 23.71 (t, J=125 Hz, C-7), 31.33 (t, J=125 Hz, C-6), 40.43 (t, J=125 Hz, C-5), 66.37 (t, J=125 Hz, CH_2 (Et)), 69.27 (t, J=125 Hz, C-1), 129.10 (d, J=158 Hz, C-3), 142.19 (d, J=158 Hz, C-2), 200.55 (s, C-4).