A Short and Convenient Synthesis of [1-^{18}O] and [4-^{18}O] Vitamin K_1

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SUMMARY

Vitamin K_1 was quantitatively reduced by $Na_2S_2O_4$ to dihydrovitamin K_1 (**2**). The hydroquinone **2** was partially methylated by CH_3I/K_2CO_3 to give a mixture of **1** and **4** monomethylethers which were separated by HPLC. Each monoether was oxidized by ceric ammonium nitrate in the presence of [^{18}O] H_2O to afford the specifically labelled [1-^{18}O] or [4-^{18}O] vitamin K_1 with isotopic enrichments of 93-95%.

Key words: [^{18}O] labelling, [1-^{18}O] phylloquinone, [4-^{18}O] phylloquinone

INTRODUCTION

Vitamin K_1 (**1**) is a cofactor which plays a key role in blood-clotting and in the photosynthesis of plants. Its labelling with ^{18}O affords a useful probe to study these biological processes [1]. In our laboratory studies [1a] [1b], structured towards the characterisation of photosynthetic reaction centers by FTIR, we have developed a short and convenient synthesis of vitamin K_1 labelled with ^{18}O at 1- or 4-positions.

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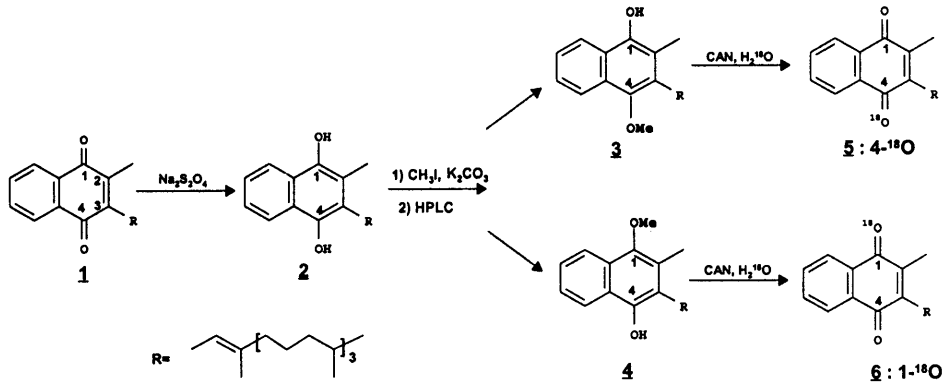
The procedure for the specific labelling of **1** with ^{18}O on the carbonyl groups is based on the oxidation reaction of the monomethylether from which the methoxy group is specifically replaced by ^{18}O in a ceric ammonium nitrate (CAN)/[^{18}O] H_2O mixture, a convenient synthesis of quinones from hydroquinones first described by N. Castagnoli and al.[2]. However, this method when applied to the labelling of **1** was rather lengthy since it requires several steps and a series of protection/deprotection procedures to prepare 1-O and 4-O- methyls which are air-sensitive [3]. Our synthesis is shorter and based on the preparation of a mixture of two monomethylethers **3** and **4** by the O-methylation of dihydrovitamin K_1 **2**, followed by their HPLC separation.Their oxidation with CAN/[^{18}O]H_2O affords **5** and **6** (scheme 1).



Scheme 1

Vitamin K_1 (**1**) was quantitatively reduced by sodium dithionite in an Et_2O/H_2O mixture to the hydroquinone (**2**). The reduced **2** was methylated by CH_3I/ K_2CO_3 in acetone to give a mixture of monoethers **3** and **4**. The HPLC purification gave

pure **3** (40%) and **4** (60%). Each monoether **3** or **4** was oxidized by CAN in a mixture of $CH_3CN/[^{18}O]$ H_2O to obtain, after HPLC purification, respectively [4-^{18}O] vitamin K_1 (**5**) and [1-^{18}O] vitamin K_1 (**6**) with isotopic enrichments of 93% and 95% respectively.

The labelling position was determined by ^{13}C NMR spectroscopy by comparison of the values obtained in the difference of the chemical shifts of the two carbonyls of **5** or **6** ($\Delta\delta^*$) with those of vitamin K_1 ($\Delta\delta$). Thus, as δ ^{18}O=C shifts to the lower fields [4] and the chemical shifts of carbonyls of **1** are known [3], for labelling at the 1-position $\Delta\delta^* < \Delta\delta$ corresponding to compound **5** and for the 4-position $\Delta\delta^* > \Delta\delta$ corresponding to compound **6**.

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EXPERIMENTAL

GENERAL

Vitamin K_1 was purchassed from Aldrich. $[^{18}O]$ H_2O (isotopic enrichment: 97%) was obtained from Eurisotop France. The HPLC analysis were performed on a Merck system and the HPLC purifications on a Dupont system. ^1H NMR spectra were recorded at 300 MHz and ^{13}C NMR at 75 MHz on a Brucker AM 400 spectrometer with $CDCl_3$ as solvent. Prior to reaction the solvents were deoxygenated by bubbling argon and the reactions were performed under an argon atmosphere.

2-Methyl-3-phytyl-1,4-naphtoquinole; dihydrovitamin K_1 (2):

The procedure described in [5] was adapted. (**1**) (275 mg, 0.61 mmol) was dissolved in diethyl ether (10 mL) and added to a 10% $Na_2S_2O_4$ solution (4 g, 23 mmol). The biphasic mixture was vigorously stirred until complete discharge of color. The reaction mixture was extracted with diethyl ether (2x10 mL) and the organic extracts were dried over anhydrous Na_2SO_4. The organic phase was evaporated in vacuo to give **2** as a white-pink solid in quantitative yield. ^{1}H NMR: 0.84-0.87 (m, 12H,CH_3 chain), 1.0-1.5 (m, 19H, CH and CH_2), 1.85 (s, 3H, CH_3-3'), 2.01 (t, 2H, CH_2-4'), 2.35 (s, 3H, CH_3-2), 3.53 (d, 2H, CH_2-1', $J_{1',4'}$ = 6.7 Hz), 4.82 (broad s, 1H, OH-4), 5.19 (dd,1H, CH-2'), 5.22 (s broad, 1H, OH-1) 7.43 (m, 2H, H-6,H-7), 8.01, 8.09 (2 m, 2H, H-8,H-5).

Methylation of 2:

2 (300 mg, 0.662 mmol) was dissolved in acetone (10 mL) and added to CH_3I (0.04 mL, 0.642 mmol) and K_2CO_3 (730mg, 5.3 mmol). After stirring for 48h at 20°C, 10 mL of water was added and the mixture extracted with diethyl ether (3x5 mL). The combined extracts were dried over saturated NaCl (2x10 mL) and evaporated to dryness. The crude oil was dissolved in a mixture of pentane/ethyl acetate: 99.25:1.75 as eluent and injected onto a silicagel column (Zorbax: 21.2 mm x 25 cm) eluted at a flow rate of 15 mL/min. The fractions appearing between 19-23 min and 38-44 min were collected and evaporated to give the monoethers **3** and **4** respectively, as colorless oils.

3: TLC: silicagel: hexane/ethyl acetate: 95:5 rf: 0.36. ^1H NMR: 0.84-0.87 (m, 12H, CH_3 chain), 1.0-1.5 (m, 19H, CH and CH_2), 1.86 (s, 3H, CH_3-3'), 2.02 (t, 2H, CH_2-4'),2.40 (s, 3H, CH_3-2), 3.53 (d, 2H, CH_2-1'), 3.84 (s, 3H, CH_3O-4), 5.22 (dd, 1H, CH-2'), 5.53 (s, 1H, OH-1), 7.43 (m, 2H, H-6,H-7), 8.01, 8.09 (2 m, 2H, H-8,H-5).

4: TLC: silicagel: hexane/ethyl acetate: 95:5 rf: 0.26. ^1H NMR: 0.84-0.87 (m, 12H, CH_3 chain), 1.0-1.5 (m, 19H, CH and CH_2), 1.80 (s, 3H, CH_3-3'), 1.96 (t, 2H, CH_2-4'),2.31 (s, 3H, CH_3-2), 3.53 (d, 2H, CH_2-1'), 3.85 (s, 3H, CH_3O-1), 4.91 (s, 1H, OH-4), 5.22 (dd, 1H, CH-2'), 7.39-7.47 (m, 2H, H-6, H-7), 7.98-8.09 (m, 2H, H-8, H-5).

[4-^{18}O] Vitamin K_1 (5):

3 (10 mg) was dissolved in CH_3CN (350 μL) and added to a CAN solution (170 mg, 0.31 mmol) in [^{18} O] H_2O (100 μL). The reaction mixture was stirred at 20°C for 30 min and extracted with a mixture of hexane/ethyl acetate: 90:10 (2x3 mL). The organic extracts were evaporated and dissolved in a mixture of pentane/ ethyl acetate: 98.75:1.75 as eluent.The HPLC purification on a silicagel column eluted at a flow rate of 25 mL/min and gave 6.9 mg of **3** (fraction collected between 6-8 min).

HRMS: calcd. for $C_{31}H_{46}{}^{16}O^{18}O$: 452.3539, found: 452.3609. EI-MS: isotopic enrichment: 93%. ^{13}C NMR: $\Delta\delta^*_{(16O=C\ -\ 18O=C)}$ = 73.2 Hz. (vitamin K_1: ^{13}C NMR: $\Delta\delta$ = 68.3 Hz).

[1-^{18}O] Vitamin K_1 (6):

The compound was prepared by the same procedure as described above. From 17 mg of **4**, 10.4 mg of **6** were obtained. HRMS: calcd. for $C_{31}H_{46}{}^{16}O^{18}O$: 452.3539, found: 452.3543. EI-MS: isotopic enrichment: 95%. ^{13}C NMR: $\Delta\delta^*_{(18O=C\ -\ 16O=C)}$ = 64.3 Hz.

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