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Research Article

Synthesis of [3′-^{14}C] coenzyme Q_{10}

Kimio Hamamura[1], Isao Yamatsu[1], Norio Minami[1], Yoji Yamagishi[1], Yuichi Inai[1], Shizumasa Kijima[1] and Tetsuya Nakamura[2]*
[1] *Eisai Research Laboratories, Eisai Co., Ltd., 4-6-10 Koishikawa, Bunkyo-ku, Tokyo 112-8088, Japan*
[2] *Department of Chemistry, Shibaura Institute of Technology, 307 Tameihara, Hukasaku, Saitama-shi, Saitama 330-8570, Japan*

Summary

Radio-labelled coenzyme Q_{10}, labelled at the 3′-position with ^{14}C, was synthesized starting from natural solanesol and ethyl [3-^{14}C] acetoacetate. The radiochemical yield was 8.0% from ethyl [3-^{14}C] acetoacetate. The specific radioactivity of the product was 44.8 μCi, 1.66 MBq/mg. The specific radioactivity and radiochemical purity are sufficiently high to enable us to use this labelled form of coenzyme Q_{10} in metabolic studies. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: [3′-^{14}C]coenzyme Q_{10}; ethyl [3-^{14}C]acetoacetate; solanesol; [3-^{14}C]decaprenol; triethyl phosphonoacetate

Introduction

Radio-labelled coenzyme Q_{10} (Co Q_{10}) is a particularly useful tool in the investigation of the metabolic fate of Co Q_{10}. This is because, while Co Q_{10} exists endogenously, exogenous Co Q_{10} has been reported to have beneficial effects under various conditions of oxidative stress. Therefore, it is advantageous to be able to differentiate the source of Co Q_{10} in vivo when discussing the role of exogenous Co Q_{10}. The number of

*Correspondence to: T. Nakamura, Vitamin E Information Section, Eisai Co., Ltd, 4-6-10 Koishikawa, Bunkyo-ku, Tokyo 112-8088, Japan.

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isoprenoid units in the side chain of Co Q homologues occurring in animals differs among species.[1] The homologue that occurs in man is Co Q_{10} (ubidecarenone).[2] The side chain has been confirmed to be of the all-*trans* configuration.[3] Using [3′-^{14}C]Co Q_{10} **6**, the behaviour of exogenous Co Q_{10} has been reported in several papers.[4–10] However, the preparation procedure of the compound has not previously been described. The purpose of this paper is to describe a detailed preparation procedure for [3′-^{14}C]Co Q_{10} **6**.

Materials and methods

Solanesol (natural) was obtained from Nisshin Flour Milling Co. (Tokyo, Japan). Ethyl [3-^{14}C]acetoacetate (specific activity 50 mCi, 1.85 GBq/mmol) was purchased from Amersham International plc (Buckinghamshire, UK). Co Q_{10} was supplied by Eisai Co. Ltd. (Tokyo, Japan). Silica gel for chromatography, Wakogel C-200, was purchased from Wako Chemical Industries, Osaka, Japan. Silica alumina was purchased from Nikki Chemical Co. (Yokohama, Japan). Thin-layer chromatography (TLC) was performed on plates of Silica Gel GF_{254} (Art 5715, Merck A. G., Darmstadt, Germany). The thickness of the plates used for analysis was 0.25 mm. Lithium aluminium hydride ($LiAlH_4$) was purchased from Merck A. G. Radioactivity was measured using a liquid scintillation counter, Aloka LSC-652 (Nihon Musen, Tokyo, Japan). Counting efficiency was determined by external standardization. All results were calculated as disintegrations per min, and all determinations were performed twice. Radioactivity on TLC plates was detected using a thin-layer chromatogram scanner, Aloka TLC-2B (Nihon Musen, Tokyo, Japan).

Results and discussion

Position 3′ of the side chain was selected for ^{14}C labelling because of the metabolic stability of this position. No metabolite of Co Q homologues has been identified with less than five carbon atoms in the side chain. The metabolic pathway of Co Q_{10} is considered to proceed via ω-oxidation and repeated β-oxidation and finally to the so-called Q acid-I, which has seven carbon atoms in its side chain, and Q acid-II, which has five carbon atoms in its side chain,[10] just as is seen in vitamin

K metabolism.[11] Metabolic studies of Co Q_{10} have been carried out in rats[12], rabbits[12] and guinea pigs.[10] The synthesis of [3′-^{14}C]Co Q_{10} **6** was performed starting from natural solanesol (**1**, all-*trans* configuration) and ethyl [3-^{14}C]acetoacetate. The total synthetic process, which includes five steps, is shown in Chart 1.

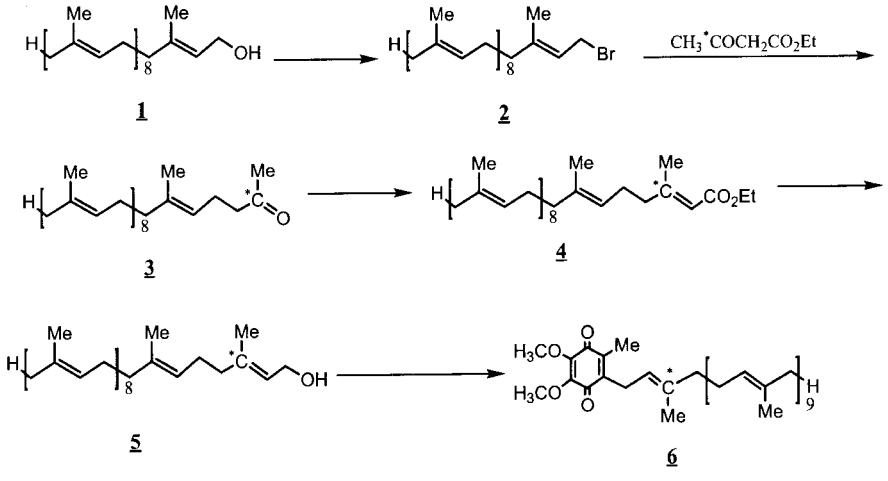

Chart 1. Synthesis of [3′-^{14}C] Coenzyme Q_{10}

Treatment of natural solanesol **1** with phosphorus tribromide followed by reaction of the bromide **2** with ethyl [3-^{14}C]acetoacetate gave [carbonyl-^{14}C]solanesyl acetone **3**. Wittig–Horner reaction of **3** with triethyl phosphonoacetate gave ethyl [3-^{14}C]decaprenoate **4**. The reaction was employed as it is known to give highly stereoselective formation of alkenes with the E-configuration.[13,14] The elongated alkene **4** was reduced with $LiAlH_4$ to give [3-^{14}C]decaprenol **5**. Condensation of **5** with Co Q_0 hydroquinone borate followed by hydrolysis and oxidation with lead dioxide gave a *cis–trans* mixture of [3′-^{14}C]Co Q_{10} **6**. The crude product was purified by chromatography on silica gel then recrystallized from cooled acetone. The overall radiochemical yield starting from ethyl [3-^{14}C]acetoacetate was 8.0%. The final product **6** was postulated to contain 1.5% of *cis*-isomer based on analysis on high-performance liquid chromatography of the corresponding deuterium-labelled compound ([2-CD_3-1′-CD_2]Co Q_{10}) synthesized by the same procedure.[15,16]

Experiments

Solanesyl bromide **2**

Solanesol **1** 6.3 g (10 mmol) was dissolved in a mixture of 0.3 ml of pyridine, 10 ml of *n*-hexane and 5 ml of ether and maintained at 5°C. A solution of phosphorus tribromide (0.6 ml) dissolved in 3 ml of *n*-hexane was added dropwise to the mixture over a two-hour period. During this time, the mixture was stirred and maintained at 5°C. The disappearance of the solanesol was checked by TLC. The reaction mixture was filtered. To the filtrate 50 ml of iced water was added and extraction was carried out with 100 ml of *n*-hexane. The extract was dried with sodium sulphate and evaporated *in vacuo*. Crude solanesyl bromide **2** 6.7 g was obtained. The crude product thus obtained was used in the next reaction without further purification.

[Carbonyl-^{14}C]Solanesyl acetone **3**

Solanesyl bromide **2** 714 mg (1 mmol) was dissolved in a mixture of ethyl [3-^{14}C]acetoacetate, 132 mg (1 mmol, 50 mCi), and 2 ml of anhydrous dioxane and maintained at 10–15°C under stirring. Freshly prepared sodium ethoxide (from 30 mg of sodium and 1 ml of ethanol) was added dropwise to this solution. After completing the addition, the reaction mixture was stirred for further 2 h. Then the reaction mixture was warmed to 80°C and 2 ml of 10% sodium hydroxide solution was added. The reaction mixture was then stirred for further 4 h. The reaction mixture was poured onto 50 ml of iced water and extracted twice, each time with 50 ml of ether. The extract was washed with water and dried over sodium sulphate. The solvent was evaporated *in vacuo*. The crude product was purified by silica gel (25 g, 60–80 mesh) chromatography. Product **3** was observed as a single peak on TLC using a radio isotope (RI) scanner. R_f value: 0.47 (*n*-hexane/EtOAc, 9:1); 0.38 (benzene). Yield: 309 mg, 20 mCi (specific radioactivity: 64.7 μCi/mg). Radiochemical yield: 40%.

Ethyl [3-^{14}C]decaprenoate **4**

Ethanol was completely removed from freshly prepared sodium ethoxide, 136 mg *in vacuo*, and 5 ml of benzene was added to the residue. To this suspension, Wittig reagent (triethyl phosphonoacetate)

452 mg (2 mmol) was added while the suspension was stirred and maintained at below 10°C. Stirring was continued until a clear solution was obtained. This solution was added dropwise to the product of the second step, [carbonyl-^{14}C]solanesyl acetone **3** 309 mg (0.46 mmol, 20 mCi) in benzene solution. After completion of the addition, the mixture was stirred for further 4 h below 10°C. The end of the reaction was recognized by the disappearance of solanesyl acetone **3** as examined by TLC. Water was added to terminate the reaction. The product was extracted with *n*-hexane. The solvent was evaporated *in vacuo*. The oil residue (290 mg) was purified by silica gel (25 g) chromatography with *n*-hexane/isopropyl ether solvent system. The product **4** was observed as a single peak on TLC using a RI scanner. R_f value: 0.51 (*n*-hexane/EtOAc, 9:1); 0.48 (*n*-hexane/isopropyl ether, 9:1). Yield: 225 mg, 16.4 mCi (specific radioactivity: 59.6 μCi/mg). Radiochemical yield: 82%.

[3-^{14}C]Decaprenol **5**

Lithium aluminium hydride 20 mg (0.5 mmol) was suspended in absolute ether. To this suspension, ethyl [3-^{14}C]decaprenoate **4** 225 mg (0.30 mmol, 13.4 mCi) was slowly added. The reaction mixture began to reflux and bubble. The mixture was allowed to react for 2 h under stirring and refluxing. After the disappearance of ethyl decaprenoate **4** was confirmed by TLC, EtOAc and then water were added to decompose the excess of $LIAlH_4$. The product was extracted with ether. The extract was evaporated *in vacuo*. The oil residue 217 mg was purified by silica gel chromatography. Product **5** was observed as a single peak on TLC using a RI scanner. R_f value: 0.26 (*n*-hexane/EtOAc, 4:1). Yield:205 mg, 12.4 mCi. (specific radioactivity: 60.5 μCi/mg). Radiochemical yield: 93%.

[3′-^{14}C]Coenzyme Q_{10} **6**

Co Q_0 hydroquinone borate was prepared by allowing a mixture of Co Q_0 hydroquinone (2-methyl-5,6-dimethoxy-1,4-hydroquinone) 221 mg (1.2 mmol), boric acid 72 mg (1.2 mmol) and 3 ml of toluene to react under stirring and refluxing. The toluene was then removed *in vacuo* and 2 ml of benzene was added to the residue to substitute the solvent. Silica alumina (400 mg) and 3 ml of *n*-hexane were added dropwise to [3-^{14}C]decaprenol **5** 205 mg (0.29 mmol, 12.4 mCi)

at 40–45°C under stirring. After completion of addition, the reaction was allowed to continue for 2 h at the same temperature. The end of the reaction was recognized by the formation of Co Q_{10} as examined by TLC. The reaction mixture was then filtered. The filtrate was oxidized by lead dioxide–acetic acid. The crude product was purified by silica gel chromatography and crystallized by addition of a 10-fold excess of cooled acetone (−2°C) compared to the crude product, and recovering the formed crystals. The final product was obtained as a yellow crystalline powder. The product **6** was observed as a single peak on TLC using a RI scanner. R_f value: 0.40 (benzene); 0.40 (*n*-hexane/EtOAc 4:1). Yield: 73 mg, 3.27 mCi (specific radioactivity: 44.8 μCi, 1.66 MBq/mg). Radiochemical yield: 26%.

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