Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVII, No. 11

SYNTHESIS OF ALL TRANS [3′ -^{14}C] MENAQUINONE-4

K. Shimada, K. Tadano[+]*, T. Satoh, K. Hashimoto, S. Tanaka and T. Yuzuriha[+]

Tokyo Research Laboratories, Eisai Co., Ltd.,
6-10, Koishikawa 4-chome, Bunkyo-ku, Tokyo 112, Japan

[+]) Tsukuba Research Laboratories, Eisai Co., Ltd.,
1-3, Tokodai 5-chome, Tsukuba-shi, Ibaraki 300-26, Japan

Summary

A synthetic procedure for producing all trans [3′ -^{14}C] menaquinone-4 is described. The radiolabel is introduced using ethyl [3-^{14}C] acetoacetate as shown in the scheme. The final product has high chemical and radiochemical purity with a specific activity of 669 MBq/mmol. The overall radiochemical yield is 12 %.

Key words : Carbon-14, Ethyl [3-^{14}C] acetoacetate, Vitamin K, [3′ -^{14}C] Menaquinone-4

Introduction

All trans menaquinone-4 [1] is one of the naturally occurring menaquinone analogues and is potentially useful for therapy of hypoprothrombinemia due to vitamin K deficiency [2]. This paper describes the synthesis of all trans [3′ -^{14}C] menaquinone-4 (11) for use in metabolism and drug disposition studies in animals [3].

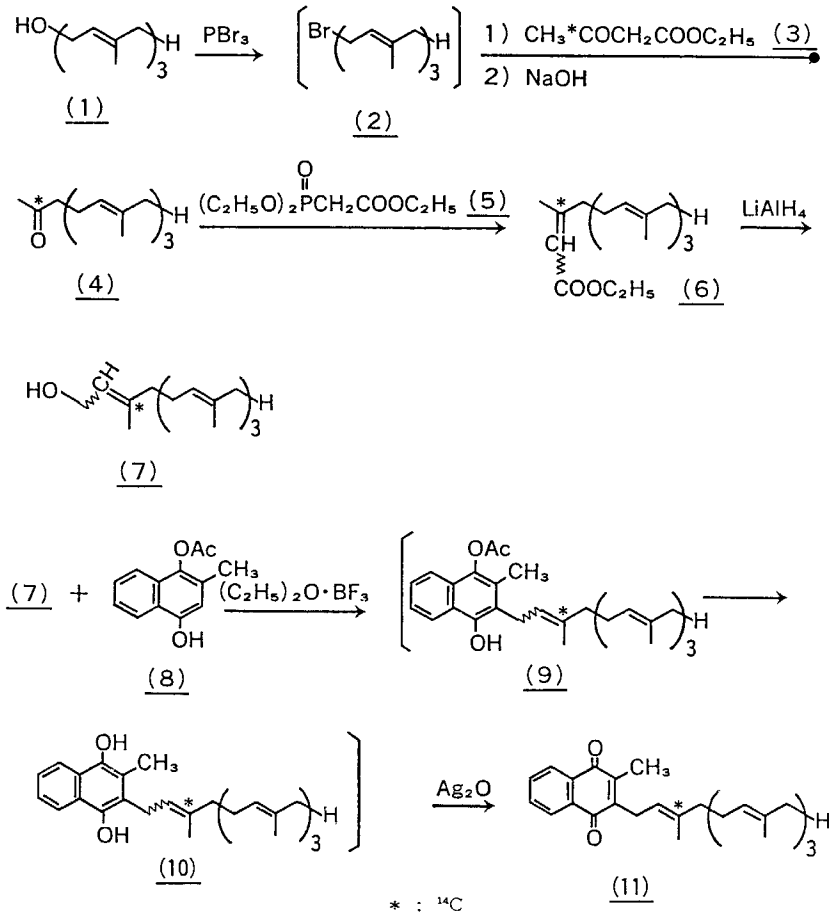
*To whom correspondence should be addressed.

0362-4803/89/111293-06$05.00

Received May 22, 1989

Results and discussion

The synthetic sequence we used is shown in the reaction scheme.



Scheme

Treatment of farnesol (1) with phosphorous tribromide followed by reaction of the bromide product [4] with ethyl [3-^{14}C]acetoacetate (3) gave [3-^{14}C]farnesylacetone (4).

Wittig reaction [5] of (4) with triethyl phosphonoacetate (5) gave the farnesylcrotonic derivative (6) which was reduced with lithium aluminum hydride to [3-^{14}C]geranylgeraniol (7).

Condensation of (7) with menadiol monoacetate (8) followed by hydrolysis and oxidation with silver oxide gave a cis-trans mixture of [3'-^{14}C]menaquinone-4 (11). The mixture was purified by chromatography on silica gel then recrystallization from acetone at -60 ℃ to give all trans [3'-^{14}C]menaquinone-4 (trans isomer ≧ 96 %). The overall radiochemical yield of this synthesis was 12 % from (3).

Preliminary experiments were carried out using unlabeled materials to optimise reaction conditions. The identity of the final product and labeled intermediates were confirmed by comparison of their TLC properties with those of the unlabeled authentic samples.

Experimental

Ethyl [3-^{14}C]acetoacetate was obtained from Amersham Japan Co., Ltd..

Radiochemical purities were determined by TLC on Kieselgel 60 F_{254} (Merck) utilizing a Berthold LB2832 automatic TLC linear analyzer in the following solvent systems, 1) acetone- n-hexane (1:1, v/v), 2) chloroform- n-hexane (1:1, v/v), 3) benzene only. HPLC analyses were performed on a system consisting of a YMC-A-012 SIL column (6 × 150 mm), a Waters pump (Model 590), a Jasco UV detector (UVIDEC-100-V, 254 nm) and a TOSOH radio LC detector (RS 8000) using a mobile phase of isopropylether- n-hexane (3:97, v/v). The t_R values for the radioactivity of cis and trans [^{14}C]menaquinone-4 were 12.1 min and 13.5 min at a flow rate of 1.0 ml/min, respectively. The radioactivity was measured with a liquid scintillation counter (LSC-903, Aloka). The quenching level was corrected by employing an automatic external standard method.

[Carbonyl-^{14}C] Farnesylacetone (4)

Phosphorous tribromide (1.17 g, 4.34 mmol) was added to a solution of farnesol (1) (1.93 g, 8.67 mmol), pyridine (0.17 g, 2.17 mmol) and n-hexane (20 ml) at 5 ℃. After being stirred at room temperature for 1 hr, the mixture was washed with methanol-water (4:1) (10 ml × 3), dried and concentrated in vacuo to give crude bromide (2) (2.43 g, oil). To a mixture of (2) (2.43 g), dioxane (10 ml) and ethyl [3-^{14}C] acetoacetate (1.26 g, 9.54 mmol) (6.66 GBq, 688 MBq/mmol) was added a solution of sodium methoxide (0.52 g, 9.54 mmol) in ethanol (5ml) at 15-20 ℃ and stirred at room temperature for 3 hr. Sodium hydroxide (1.0 g, 25 mmol) in water (10 ml) was added, and the mixture was refluxed for 2 hr. After cooling, n-hexane (20 ml) was added, the separated organic layer was washed with methanol-water (3:1) (10 ml × 3), dried and concentrated in vacuo to give crude (4) (oil). This was purified by chromatography on silica gel, eluting with 2-5 % isopropylether in n-hexane, to give (4), 1.39 g (66.1 %) (colorless oil).

Ethyl [3-^{14}C] (3-methyl-4-farnesyl) crotonate (6)

A solution of (4) (1.39 g, 5.3 mmol) in n-hexane (30 ml) was added to a mixture of triethyl phosphonoacetate (5) (2.4 g, 10.6 mmol), sodium ethoxide (0.72 g, 10.6 mmol) and n-hexane (30 ml) at 15 ℃, and stirred at room temperature for 4 hr. The mixture was washed with methanol-water (2:1) (10 ml × 3), separated and the hexane layer was evaporated in vacuo to give crude (6) (1.76 g) (colorless oil).

[3-^{14}C] Geranylgeraniol (7)

A solution of crude (6) (1.76 g), ether (30 ml) and lithium aluminum hydride (0.2 g, 5.3 mmol) was stirred at room temperature for 1 hr. Water (few drops) was added, and the mixture was

filtered. The filtrate was concentrated in vacuo to give crude (7) (oil). This was purified by chromatography on silica gel, eluting with 5 % isopropylether in n-hexane, to give 1.39 g (90.3 %, calculated from (4)), (colorless oil).

[3'-^{14}C]Menaquinone-4 (11)

To a solution of menadiol monoacetate (8) (2.3 g, 10.6 mmol) in dioxane-ethylacetate (1:1) (20 ml) was added boron trifluoride-etherate (330 μl, 2.68 mmol) at 40-50 ℃, and then (7) (1.39 g, 4.8 mmol) was added slowly, stirred for 3 hr at the same temperature. After cooling, isopropylether (10 ml) was added, and the mixture was washed with water (5 ml × 2), then dried. The solvent was evaporated in vacuo to give crude (9) (oil).
A mixture of crude (9), Claisen's alkali solution *) (10 ml) and sodium hydrosulfite (1.0 g, 5.7 mmol) was stirred at room temperature for 0.5 hr. Isopropylether (10 ml) was added, the isopropylether layer was neutralized with acetic acid and washed with water (5 ml × 2).
To this isopropyl solution was added silver oxide (1.4 g, 6 ml), and the mixture was stirred at room temperature for 45 min. The mixture was filtered, then the filtrate was evaporated to dryness. The residue was chromatographed on silica gel, eluting with 5 % isopropylether in n-hexane, to give a cis-trans mixture of (11) (about 700 mg).
Two recrystallizations from acetone (7 ml) at -60 ℃ gave 514 mg of (11) (21.5 %, calculated from (4)) (yellow crystals) of specific activity 669 MBq/mmol (40.55 μCi/mg) and radiochemical purity, as determined in various TLC systems, as follows, system 1) ≧ 99 %, system 2) ≧ 99 % and system 3) ≧ 98 %. The trans isomer was shown to be ≧ 96 % by HPLC analysis.

*) Claisen's alkali solution : potassium hydroxide (35 g), water (25 ml) and methanol (100 ml).

References

1) McCormic D . B . and Wright L . D . Ed. – Method in Enzymology, XVII part C, p. 407 (1971). Academic press, New York, NY.

2) Gilman A . G . , Goodman L . S . , Rall T . W . and Murad F . Ed. – The Pharmacological Basis of Therapeutics, 7 th. ed., p. 1582 (1985). MacMillan Publishing Company, New York, NY.

3) Tadano K . , Yuzuriha T . and Miyake Y . – J. Nutr. Sci. Vitaminol., (1989), in press.

4) Hauser C . R . and Hudson B . E . – Organic Reactions, 1 : 267 (1942). John Wiley & Sons, Inc., New York, NY.

5) Macker A . – Organic Reactions, 14 : 270 (1965). John Wiley & Sons, Inc., New York, NY.