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Synthesis of 2S,4'R, 8'R-α-[5-Methyl-^{14}C] Tocopheryl Acetate

T.Nakamura[1]*, K.Hamamura[2], and T. Ueda[3]

[1]Department of Chemistry, Shibaura Institute of Technology, Tameihara 307, Fukasaku, Ohmiya-shi, Saitama 330-8570, Japan

[2]Eisai Research Laboratories, Eisai Co., Ltd., 4-6-10 Koishikawa, Bunkyo-ku, Tokyo 112-8088, Japan

[3]Department of Human Nutrition, Tokyo Metropolitan Research Laboratory of Public Health, Hyakunin-cho 3-24-1, Shinjyuku-ku, Tokyo 169-0073, Japan

Address for correspondance,

Tetsuya Nakamura, Ph.D.
Vitamin E Information and Technology Section
Eisai Co.,Ltd.
Bunkyo-ku, Tokyo 112-8088, Japan
Phone No: +81-3-3817-5369, Fax:+81-3-3811-7513
email: t5-nakamura@hcc.eisai.co.jp

Summary

2-Ambo-γ-tocopheryl acetate **4** was synthesized starting from 2,3-dimethylhydroquinone monobenzoate **1** and natural phytol **2**. 2S,4'R,8'R-enantiomer **6** was separated using preparative Chiralpack OP (+) column HPLC.

Key Words: 2S,4'R,8'R-α-[5-methyl-^{14}C] tocopheryl acetate, 2-ambo-γ-tocopherol, 2S,4'R,8'R-γ-tocopherol, [^{14}C]-paraformaldehyde, chiral column HPLC

*Author for correspondence

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After hydrolysis of the acetate with $LiAlH_4$, the obtained free tocopherol **7** was reacted with [^{14}C]-paraformaldehyde under the presence of boric acid, and then reduced. Thus 2S, 4'R, 8'R-α-[5-methyl-^{14}C] tocopherol **8** was obtained. The stereospecificity (99.1 %) was checked by chiral HPLC in acetate **9**. The overall yield of **9** was 48.5 % based on [^{14}C]-paraformaldehyde.

Introduction

Vitamin E (α-tocopherol) is available in natural (2R,4'R,8'R) and synthetic (all-racemic, 2RS, 4'RS, 8'RS) forms. Due to low source supply, the synthetic form is an attractive alternative. However, as vitamin E has three asymmetric carbon centers, the all-racemic form is a mixture of eight stereoisomers (1). Eight possible stereoisomers of unlabelled α-tocopherol have been synthesized, and had the relative biopotencies evaluated with classical rat resorption-gestation tests (2). Pharmacokinetic studies on vitamin E stereoisomers have mainly been done using deuterium-labelled compounds (3). In particular, a comparison of RRR- and SRR-forms was carried out using stable isotope labelled compounds in rats (4), monkeys (5) and humans (6,7,8). In these studies SRR-α-[5-CD_3]tocopherol, which was obtained after repeated recrystallization of a mixture of SRR- and RRR-α-[5-CD_3]tocopheryl-p-phenylazobenzoates (4) was used. In general, there is a limitation to clarifying the whole metabolic profile when employing stable isotopes since analyses of labelled compounds are restricted to only unchanged forms. The post-absorptive fate of SRR-α-tocopherol is hypothesized to be excretion in bile (9).

The purpose of this report is to further clarify this issue by providing novel radiolabelled compounds of an α-tocopherol stereoisomer. The use of radiolabelled compounds is indispensable to clearly show the differences in metabolism between RRR- and SRR-forms. The synthesis of 2R,4'R,8'R-α-[5-methyl-^{14}C]tocopheryl acetate has been reported previously (10,11), while that of the corresponding SRR-form has not. The starting material selected for synthesis

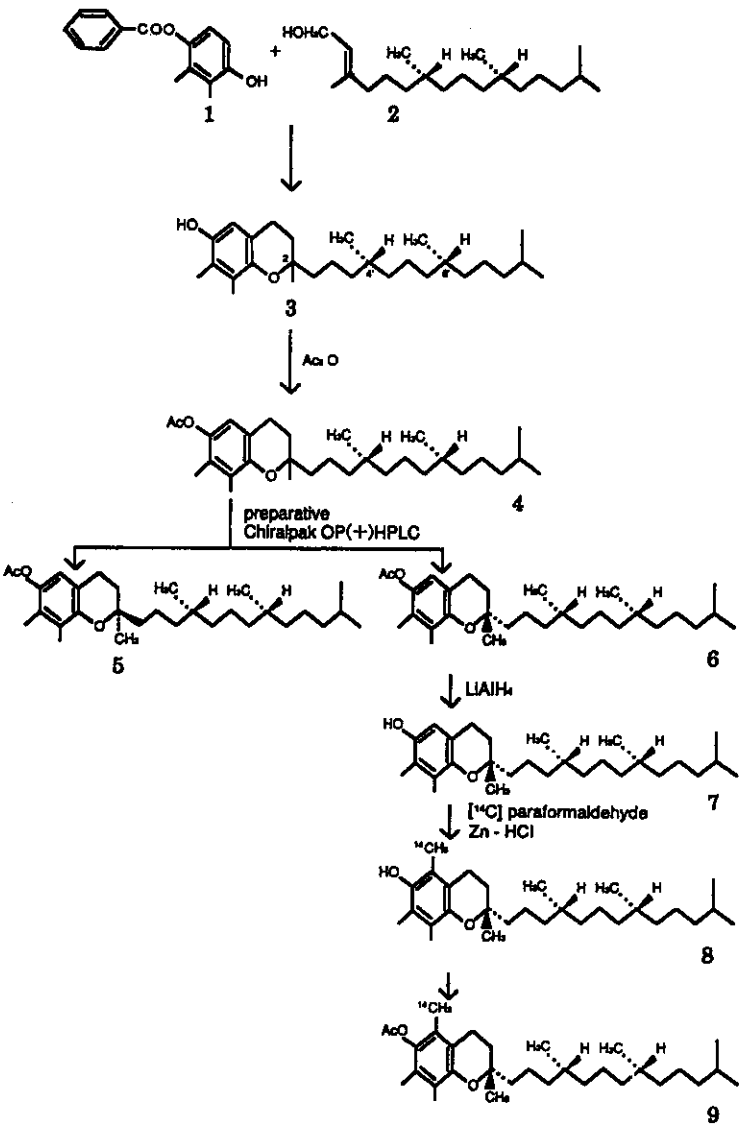
of 2S,4'R,8'R-α-[5-methyl-^{14}C]tocopherol was newly isolated 2S,4'R,8'R-γ-tocopherol, which was obtained by separation of ambo-γ-tocopherol (10) using a previously described chiral chromatographic procedure (12). A methyl group was introduced at the 5-position of γ-tocopherol by hydroxymethylation followed by reduction (11). Thus 2S,4'R,8'R-α-[5-methyl-^{14}C]tocopheryl acetate **9** was synthesized via a practical synthetic route.

Results and Discussion

The synthesis of 2S,4'R,8'R-α-[5-methyl-^{14}C]tocopheryl acetate **9** was performed as shown in Scheme 1. Natural phytol **2** and 2,3-dimethylhydroquinone monobenzoate **1** were condensed, and 2-ambo-γ-tocopherol **3** was obtained (10). After acetylation of **3**, 2S,4'R,8'R-γ-tocopheryl acetate **6** was isolated by preparative Chiralpak OP (+) column HPLC. Preparative chiral column separation of ambo-γ-tocopherol was done by modifying a procedure established previously (12). The stereospecific purity of **6** was 99.7% on HPLC. 2S,4'R,8'R-γ-tocopherol **7** was obtained after hydrolysis of **6** with $LiAlH_4$. **7** was hydroxymethylated with [^{14}C]-paraformaldehyde with a boric acid catalyst and immediately reduced to 2S,4'R,8'R-α-[5-methyl-^{14}C]tocopherol **8**. The final confirmation of stereospecificity was done by chiral column HPLC in acetate **9**. It has been shown that the absorption ratio of RRR- and SRR-α-tocopherol is the same until the chylomicron fraction in plasma (5). However, contrary to RRR-α-tocopherol, information on the post-absorptive metabolic fate of SRR-α-tocopherol is relatively sparse (9). Recently, the affinity of SRR-α-tocopherol to α-tocopherol transfer protein, which is supposed to distinguish between vitamin E related compounds, was found to be 11% of RRR-α-tocopherol on in vitro experiment (13).

Conclusion

This study provided a practical route to obtaining a carbon-14 labelled α-tocopherol stereoisomer. A comparative catabolic study using 2R,4'R,8'R- and 2S,4'R,8'R-α-[5-methyl-^{14}C]tocopherol is underway.



Scheme 1.

Experimental

General

Natural phytol was obtained from Tama Biochemical Co. (Tokyo, Japan). [^{14}C]-Paraformaldehyde was purchased from Amersham Co. (UK). Thin layer chromatography (TLC) was carried out with Merck 5417 (Darmstadt, Germany).

High performance liquid chromatography (HPLC) system Model LC-6AD Series from Shimadzu Manufacturing Co. (Tokyo, Japan) consisting of an SIL-9A automatic injector, CTO-6A column oven, LC-6AD pump, and SPD-6AV UV detector linked to a C-R6A integrator was used. The HPLC analyses were carried out at 35°C, and ultraviolet detection was at 284 nm without any modification. Nuclear magnetic resonance (NMR): AM200, Brucker (Conventry, UK), solvent, $CDCl_3$ (d-chloroform). Mass spectrum (MS) [for unlabelled compounds]: JMX-HX100, Mass Spectrometer, JEOL (Tokyo, Japan); technique, FAB^+; FAB matrix, m-nitrobenzyl alcohol; FAB gas, Xenon (accelerating voltage 6kV); mass range, 100-1100; scan rate, 11.0 sec/scan; sample preparation, sample dissolved in methanol, and applied to probe tip with FAB matrix. MS [for labelled compounds]: VG Trio 2000 Mass Spectrometer, VG Biotech (Cheshire, UK), technique; DCI^+, reagent gas; NH_3, mass range; 50-600 amu, scan rate; 0.8 sec/scan, probe gradient; 0-1500mA at 5000mA/sec, sample preparation; sample dissolved in ethanol ~1mg/ml, 2 μl applied to probe tip. The calculation of specific activity for carbon-14 labelled compound was done from mass spectra data (14).

Synthesis

2,3-Dimethylhydroquinone monobenzoate 1. To a solution of 2,3-dimethylhydroquinone (6.9 g, 50 mmol) in dry pyridine 30 ml, benzoyl chloride (8.43 g, 60 mmol) was added and the mixture was left at room temperature for 20 hrs. The crude benzoate mixture obtained was then dissolved in hot methanol. On cooling, 1.3 g of the dibenzoate precipitated from solution and crystallized in colorless needles, m.p. 182°C. After elimination of the dibenzoate, the solvent was evaporated and monobenzoate 1 was recrystallized from the residue with acetone-petroleum ether to form colorless needles; yield 7.8 g (64.5%), m.p. 174°C (15). IR (cm^{-1}): 3400 (OH), 1760 (ester). NMR ($CDCl_3$, δ): 7.30 (5H, m), 6.68 (1H, s), 6.38 (1H, s), 5.50 (1H, s), 2.10 (3H, s), 2.00 (3H,s)

(2RS,4'R,8'R)-γ-Tocopherol 3. 2,3-Dimethylhydroquinone monobenzoate 1 (4.84 g, 20 mmol), natural phytol 2 (6.23 g, 20 mmol, purity: 95%) and anhydrous

zinc chloride (2.72 g, 20 mmol) were refluxed in dry dichloromethane (50 ml) for 2 hrs in a nitrogen atmosphere. The reaction mixture was poured onto water and extracted with dichloromethane (20 ml). The extracts were hydrolyzed with 5N sodium hydroxide (20 ml) for 3 hrs at room temperature. The solution was washed with water, dried and evaporated. The residual oil was dissolved in toluene (10 ml) and chromatographed through a column of silica gel (150 g) (200 mesh) with n-hexane-isopropylether. The pure 2RS,4'R,8'R-γ-tocopherol **3** (10) was obtained as a yellowish oil. Yield 7.3 g (87.7 %). IR (cm^{-1}): 3350 (OH). NMR ($CDCl_3$, δ): 6.55 (1H, s), 2.66 (2H, t), 2.16 (3H, s), 2.08 (3H, s), 1.84 (2H,t), 1.60~1.03 (27H, m), 0.86 (3H, s), 0.83 (3H, s), 0.70 (3H, s).

(2RS,4'R,8'R)-γ-Tocopheryl acetate 4. (2RS,4'R,8'R)- γ-Tocopherol **3** (6.24 g, 15 mmol), acetic anhydride (10 ml, 110 mmol) and 4-dimethylaminopyridine (1 g, 8 mmol) were dissolved in dichloromethane (30 ml) and stirred for 3 hrs at room temperature, then poured over ice. The mixture was stirred for 30 min at 0 - 5°C, and extracted with dichloromethane (20 ml). The extracts were washed with water, dilute sodium bicarbonate solution, water again, dried and evaporated in vacuo to give a yellowish oil. Distillation under high vacuum (b.p. 195-200 °C at 0.1 mm Hg) afforded 6.1 g of (2RS,4'R,8'R)- γ-tocopheryl acetate **4** as a colorless oil. Yield: 88%. Non-chiral isocratic HPLC, column; Inertsil ODS-2 (4.6 I.D. x 250 mm), mobile phase; methanol: water (49:1 v/v), flow rate; 1.5 ml/min, detection; UV 284 nm, retention time; 11.4 min, purity; 95.2 %. Stereospecific isocratic HPLC analyses of **4** were carried out by a slightly modified procedure as described previously (12). Column; Chiralpak OP (+) (4.6 I.D. x 250 mm, particle size 10 μm, Daicel Chem. Ind., Tokyo, Japan), mobile phase; methanol: water (95:5 v/v), flow rate; 0.5 ml/min, retention time for (2R,4'R,8'R)- γ-tocopheryl acetate **5**; 12.0 min (purity 49.5%), for (2S,4'R,8'R)- γ-tocopheryl acetate **6**; 14.2 min (purity 49.5%). IR (cm^{-1}): 1760 (ester). NMR ($CDCl_3$, δ): 6.60 (1H, s), 2.66 (2H, t), 2.16 (3H, s), 2.05 (3H, s), 1.86 (2H,t), 1.60~1.05 (27H, m), 0.88 (3H, s), 0.85 (3H, s), 0.74 (3H, s). Mass spectrum (m/e): M^+ = 458, 416, 233, 193, 151.

(2S,4'R,8'R)-γ-Tocopheryl acetate 6. 2-Ambo-γ-tocopheryl acetate 4 (1.5 mg / 500 µl of 0.6 % methanol solution / injection) was injected to preparative HPLC with Chiralpak OP (+) column (20 I.D. x 250 mm, particle size 10 µm, Daicel Chem. Ind.). Mobile phase: methanol, eluting rate: 2.5 ml/min. Detection; UV 284 nm. Injections were repeated 394 times. To the combined eluate (200 ml) distilled water (250 ml) and hexane (70 ml) were added and stirred for 5 min to extract the hexane layer. The hexane extract was dried with Na_2SO_4 and evaporated under nitrogen gas to obtain 6 555 mg (Yield: 94%. Identification with HPLC was performed as follows; (i) Non-chiral HPLC, equipment, SC-8020 type (Toso Co.); column, nucleosil 5NH_2, 4.6 I.D. x 250 mm (Marcherey-Nagel Co., Germany); mobile phase, 1.6 % isopropanol/hexane, detection; retention time, 4.8 min; purity, 99.9 %. (ii) Stereospecific HPLC, equipment, SC-8020 type (Toso Co.); column, Chiralpak OP (+), 4.6 I.D. x 250 mm; column temperature, 35°C; mobile phase, methanol; detection, retention time, 21.2 min; purity, 100 %.

(2S,4'R,8'R)-γ-Tocopherol 7. To a mixture of $LiAlH_4$ (0.1 g, 2.7 mmol) in dry diethylether (5ml), a solution of (2S,4'R,8'R)-γ-tocopheryl acetate 6 (0.62 g, 1.35 mmol) in dry diethylether (5 ml) was added dropwise at 0°C for 1 hr in a nitrogen atmosphere. The mixture was stirred at 0°C for 1 hr and water (0.1 ml), 15% NaOH solution (0.1 ml), and water (0.3 ml) were added in successive drops for 30 min. The mixture of dry granular precipitate was filtered and washed with diethylether (5 ml). The ether solution was dried and evaporated in vacuo to give 525 mg of (2S, 4'R, 8'R)-γ-tocopherol 7. (Yield: 92.9%). Non-chiral HPLC purity as α-tocopherol; 98.1%.

2S,4'R,8'R-α-[5-methyl ^{14}C] tocopherol 8. To SRR-γ-tocopherol 7 (350 mg, 0.84 mmol) in toluene (4 ml), acetic acid (8 drops) and boric acid (76 mg, 1.2 mmol) were added. Paraformaldehyde (1.85 GBq, 50 mCi/28 mg, 55 mCi/mmol based on a monomer, CFA81, Nycomed Amersham Plc, UK) was added and the reaction mixture was stirred at 100 °C for 3 hrs. The reaction mixture was cooled to room temperature and toluene (50 ml) was added. The toluene layer was

washed with water (2 x 50 ml). TLC indicated that approximately 70 % of γ-tocopherol had changed to α-[5-hydroxymethyl ^{14}C]tocopherol (11). The toluene solution was used directly in the next step without further analysis. To the toluene solution, concentrated hydrochloric acid (7.5 ml), acetic acid (5 ml) and zinc dust (750 mg) were added. The reaction mixture was stirred at room temperature for 2 hrs. TLC showed that all α-[5-hydroxymethyl ^{14}C]tocopherol was reduced to α-[5-methyl ^{14}C]tocopherol. The reaction mixture was washed with water (3 x 50 ml), dilute sodium bicarbonate (2 x 50 ml) and water (3 x 50 ml). The organic solution was dried over magnesium sulphate and rotary evaporated to a thick oil. This was purified by normal non chiral HPLC. The material was again rotary evaporated to a thick oil to give **8** (203.5 mg, 25 mCi, specific activity; 122.85 μCi/mg, radiochemical yield from [^{14}C] paraformaldehyde; 50%.

2S,4'R,8'R-α-[5-methyl ^{14}C] tocopheryl acetate 9. α-[5-methyl ^{14}C] tocopherol **8** (203.5 mg, 25 mCi) was dissolved in acetic anhydride (9.6 ml) and pyridine (3.2 ml). The reaction mixture was heated at 95 °C for 2 hrs. TLC indicated product only. This was added to water (50 ml) and extracted with hexane (2 x 25 ml). The combined organics were washed with water (2 x 50 ml). The organic solution was dried over magnesium sulphate, filtered and purified by non-chiral HPLC. The material **9** was rotary evaporated and stored as an ethanolic solution. Product specification of SRR-α-[5-methyl-^{14}C] tocopheryl acetate **9** was as follows: Total radioactivity; 0.89 GBq (24.3 mCi), radiochemical yield; 48.5% (from [^{14}C]-paraformaldehyde); specific activity (determined by mass spectroscopy); 2.04 GBq/mmol (55mCi/mmol), molecular weight; 474.6 (at this specific activity), chemical purity; TLC, 99.3%, in petroleum ether (80-100): Isopropyl ether (8:2), 99.5% in hexane:ethyl acetate (5:1). Non-chiral HPLC purity: (i) 99.1%, column; YMC-PACK ODS-AM312 (6 I.D. x 150 mm) at 40°C, mobile phase; methanol: water (95:5), flow rate; 1.5ml/min, UV detection; 292 nm. (ii) 99.1%, column; NUCLEOSIL 5NH_2 (250 x 4.6mm), mobile phase; 1.6% isopropanol in hexane, flow rate; 1.0ml/min, UV detection; 268 nm. The stereospecific HPLC chromatograms of 2S,4'R,8'R-α-[5-methyl ^{14}C]tocopheryl acetate **9** and

2R,4'R,8'R- α-[5-methyl ^{14}C]tocopheryl acetate (11) are shown in Fig. 1. H-NMR($CDCl_3$): δ 0.88 (3H, s), 0.92 (6H, s), 1.05~1.6 (27H, m), 1.80 (2H, m), 1.98 (3H, s), 2.05 (3H, s), 2.10 (3H, s), 2.32 (3H, s), 2.6 (2H, t). MS was run in chemical ionization mode using ammonia as the reagent. Thus the molecular weight observed was 474 + 18, or 492.

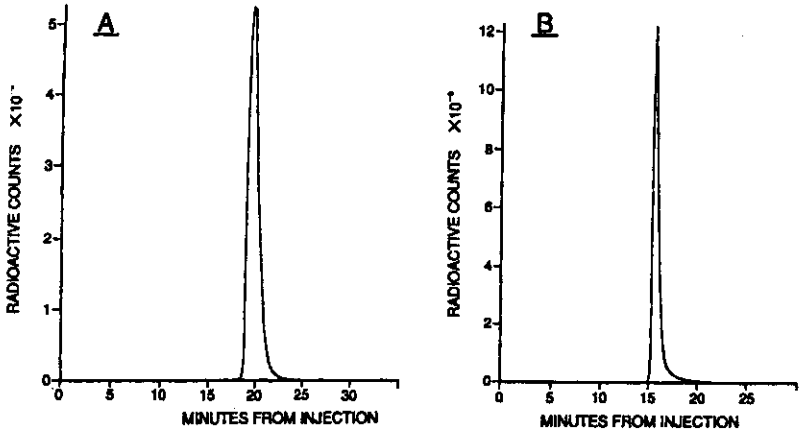


Fig. 1. Stereospecific HPLC chromatograms of (A) 2S,4'R,8'R-α-[5-methyl ^{14}C] tocopheryl acetate 9 (purity 99.1%) and (B) 2R,4'R,8'R- α -[5-methyl ^{14}C] tocopheryl acetate (purity 99.8%) (11). HPLC conditions were as follows; column, chiralpack OP (+) 4.6 I.D. x 250 mm; temperature, 35 °C; mobile phase, methanol; flow rate, 0.3ml/min; ultraviolet detection, 284 nm.

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