THE SYNTHESIS OF C-13 LABELED VITAMIN E, [12'-^{13}C]*all-rac*-α-TOCOPHEROL[1)]

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Summary

[12'-^{13}C]*all-rac*-α-Tocopherol (1) was synthesized from [2-^{13}C]acetone. The condensation of 6-methoxymethoxy-2,5,7,8-tetramethyl-2-(5-mercaptothiazolinyl-4-methyl-3-penten-1-yl)chroman (8) with [7-^{13}C]geranyl bromide (7), which was prepared by the coupling of (6-benzoyloxy--4-methyl-4-hexenyl)triphenylphosphonium bromide (5) with [2-^{13}C]acetone following bromination, afforded [12'-^{13}C]6-methoxymethoxy-2,5,7,8-tetramethyl-2-[(3E,7E,9E)-5-mercaptothiazolinyl-4,8,12-trimethyl-3,7,9-tridecatrien-1-yl)chroman (9). After desulfurization and reduction of 9, the reaction product obtained was converted into [12'-^{13}C]*all-rac*-α-tocopherol (1)by hydrolysis. The total yield of 1 was 33.7% on the basis of [2-^{13}C]acetone.

Key Words: Vitamin E, ^{13}C-Labeled α-tocopherol, synthesis, [12'-^{13}C]*all-rac*-α-Tocopherol

Introduction

Although it has been considered that α-tocopherol (vitamin E)

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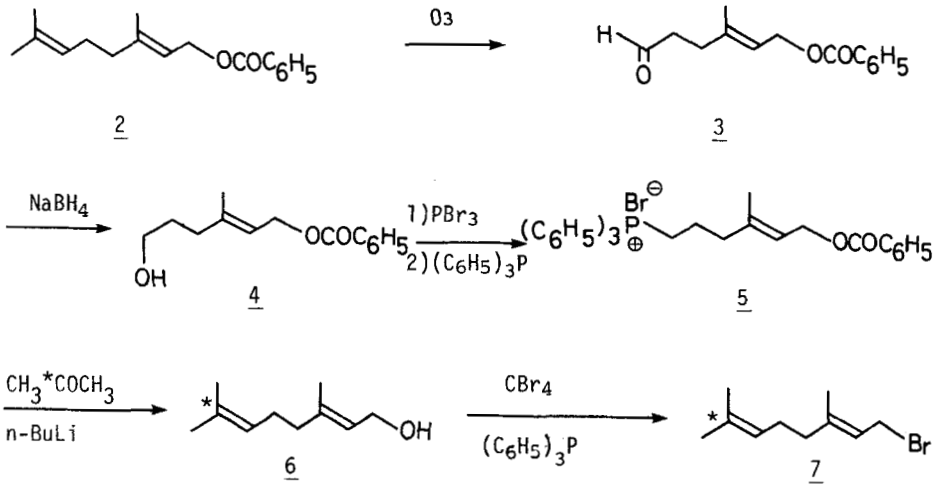
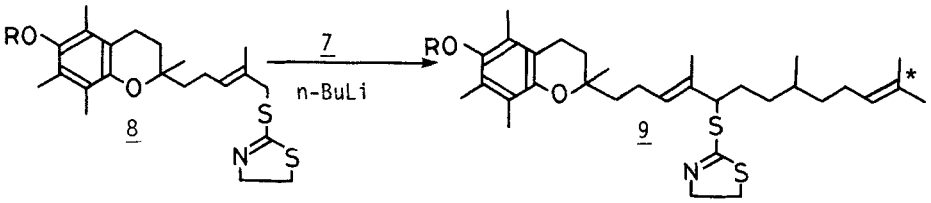
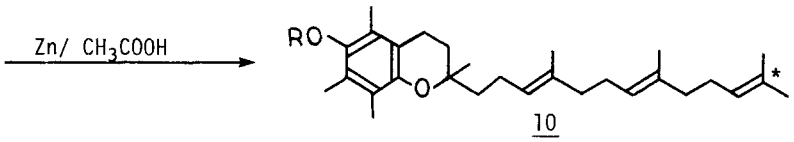
acts as an biological antioxidant and a radical scavenger[2] and also has a biomembrane stabilizing effect[3], its biological function has not yet been elucidated. In paticular, the role of its isoprenoid side chain remains obscure. Recently, interest centers in the interaction between its side chain and lipid components in biomembrane. It is considered that the use of α-tocopherol having the labeled isoprenoid moity is of great advantage to the investigation of such an interaction. However, there is report on neither the dynamic study with the use of ^{13}C-labeled α-tocopherol nor the synthesis of α-tocopherol with a labeled isoprenoid side chain. We now wish to report the synhtesis of [12'-^{13}C]*all*-*rac*-α-tocopherol (1).

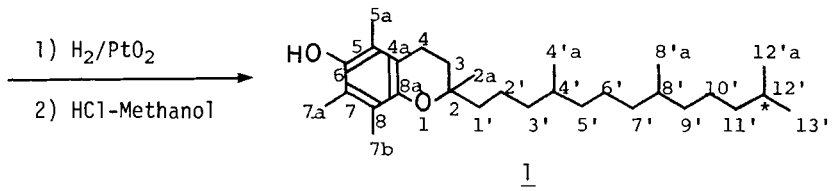
Results and discussion

The reaction sequence for the synthesis of [12'-^{13}C]*all*-*rac*-α-tocopherol (1) is illustrated as follows (asterisked carbons indicate ^{13}C).









Ozonolysis of geranyl benzoate (2) in dry methylene chloride at -78°C in a dry ice-acetone bath followed by reduction of the reaction product gave 6-hydroxy-3-methyl-2-hexenyl benzoate (4) in 94.8% yield from 2. Then, 4 was brominated with phosphorus tribromide. The bromide resulted was converted into a Wittig reagent, (6-benzoyloxy-4-methyl-4-hexenyl)triphenylphosphonium bromide (5), with triphenylphosphine in 89.4% yield. Treatment of 5 with n-butyl lithium and [2-^{13}C]acetone gave [7-^{13}C]geraniol (6) in 75.0% yield. The labeled position in 6 was confirmed spectroscopically; in the ^{13}C-NMR spectrum ($CDCl_3$) the intensity of a signal at 131.4 ppm is greatly enhanced and signals at 17.6

and 25.6 ppm are split with the coupling constants of 57.9 and 56.9 Hz, respectively. With carbon tetrabromide and triphenylphosphine, 6 was converted into [7-^{13}C]geranyl bromide (7). Because of its unstability, 7 was used in the next step without purification.

6-Methoxymethoxy-2,5,7,8-tetramethyl-2-(5-mercaptothiazolinyl-4-methyl-3-penten-1-yl)chroman, which was prepared previously[4], was reacted with the crude 7 in the presence of n-butyl lithium to give [12'-^{13}C]6-methoxymethoxy-2,5,7,8-tetramethyl-2-[(3E,7E,9E)-5-mercaptothiazolinyl-4,8,12-trimethyl-3,7,9-tridecatrien-1-yl)-chroman (9) in 78.8% yield. The ^{13}C-NMR spectrum ($CDCl_3$) of 9 shows that the intensity of a signal at 131.2 ppm is enhanced and signals at 17.7, 26.6 and 124.2 ppm are split due to ^{13}C-^{13}C coupling (J_{C-C}=41.1, 43.5 and 61.2 Hz, respectively). With zinc powder 9 was desulfurized in acetic acid to give [12'-^{13}C]-6-methoxymethoxy-2,5,7,8-tetramethyl-2-[(3E,7E,9E)-4,8,12-trimethyl-3,7,9-tridecatrien-1-yl)chroman (10) in 63.8% yield. Finally, 10 was reduced under 50 atmosphere of hydrogen in the presence of platinum oxide and reduction product was hydrolysed with a methanol solution of hydrogen chloride to give the desired [12'-^{13}C]-α-tocopherol (1) in 89.5% yield from 10. The total yield of 1 based on [2-^{13}C]acetone was 33.7% and the overall yield from 2 was 28.6%.

Experimental

^{1}H- and ^{13}C-NMR spectra were recorded on a Varian XL-200 spectrometer employing $CDCl_3$ or CD_3OD as solvents and tetramethyl silane as an internal standard. Mass, UV and IR spectra were taken with the spectrometers of Shimadzu-LKB 9000, Cary 118C and Jasco IRA-2, respectively. Gas chromatograms were obtained on a

Shimadzu GC-5A with a flame ionization detector. Silica gel PF_{254} (Merck, Darmstadt, BRD) was used for thin layer chromatography and silica gel C-200 (Wako, Osaka, Japan) for column chromatography. All other chemicals were obtained from common laboratory suppliers.

3-Methyl-6-oxo-2-hexenyl benzoate (3). Geranyl benzoate (10 g, 38.8 mmol) was dissolved into dry methylene chloride. Ozone gas was introduced into the solution at -78°C in a dry ice-acetone bath for 4 hours. After being warmed to room temperature, the reaction mixture was washed with water and dried over sodium sulfate. The solution was evaporated. The residue was purified by silica gel chromatography using benzene as an eluent to give 3 (8.5 g, 94.8%). Mass (M^+) 232; IR (neat) 1685, 1750 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ, 9.78 (bt, 1H, -CHO), 4.86 (d, 2H, J=7.5 Hz, $-CH_2-O-$), 1.76 (s, 3H, $-CH_3$); ^{13}C-NMR ($CDCl_3$) δ, 201.5 (d,-CHO), 61.6 (t, $-CH_2-$) , 16.6 (q, $-CH_3$).

6-Hydroxy-3-methyl-2-hexenyl benzoate (4). To a solution of 3 (4.0 g, 17.0 mmol) in methanol (100 ml), sodium borohydride (650 mg, 17.0 mmol) was added with stirring at 0°C. The stirring was continued for 3 hours. The reaction mixture was poured into ice water (150 ml) and extracted with ether. The ether extract was washed with brine, 10% acetic acid and water, in turn. The extract was dried and concentrated. The residue was chromatographed on a silica gel column and eluted with a mixture of benzene and ether to give pure 4 (4.0 g, 99.1%). Mass (M^+) 234; IR (neat) 3390, 1751 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ, 4.87 (d, 2H, J=7.5 Hz, $-CH_2-OCO-$), 3.66 (t, 2H, J=7.5 Hz, $-CH_2-OH$), 1.78 (s, 3H, $-CH_3$); ^{13}C-NMR ($CDCl_3$) δ, 63.1 (t, $-CH_2-O-$), 61.8 (t, $-CH_2-O-$).

(6-benzolyloxy-4-methyl-4-hexenyl)triphenylphosphonium bromide (5).

To a solution of 4 (4.0 g, 17.0 mmol) in dry chloroform (100 ml) PBr_3 (4.6 g) was added with stirring at 0°C. The stirring was continued at 0°C for 30 min. The mixture was poured into ice water and extracted with chloroform. The chloroform extract was washed with water, a saturated sodium bicarbonate and brine, in turn, and then dried over sodium sulfate. The chloroform solution was evaporated at 40°C to give the crude product (4.5 g). The residue was dissolved in dry toluene (30 ml). To this solution was added, with stirring at reflux, a solution of triphenylphosphine (3.7 g, 14.0 mmol) in dry toluene (30 ml). The reflux was continued for 15 hours. The solvent was concentrated at 80°C. The residue was triturated with cold toluene and filtered. The white solid obtained was dried in a vacuum oven at 50°C to afford 5 (7.6 g, 89.4%). IR (neat) 1750 cm^{-1}; ^{1}H-NMR (CD_3OD) δ, 4.86 (d, 2H, J=7.5 Hz, $-CH_2-O-$), 3.24 (m, 2H, J_{P-C-H}=12.0 Hz, $P-CH_2$), 1.68 (s, 3H, $-CH_3$); ^{13}C-NMR (CD_3OD) δ, 40.4 (dt, $J_{C-C-C-P}$=16.1 Hz, $-CH_2-$), 21.9 (dt, J_{C-P}=52.2 Hz, $P-CH_2-$), 21.1 (dt, $J_{C-C-C-P}$=3.6 Hz, $-CH_2-$).

[7-^{13}C]Geraniol (6). To a suspension of 5 (4.5 g, 8 mmol) in dry tetrahydrofuran(50 ml) was added with stirring at 0°C under nitrogen gas a solution of n-butyl lithium (10 ml, 1.6 mol) in dry tetrahydrofuran. The stirring was continued for 30 min at room temperature. After a solution of [2-^{13}C]acetone (0.5 g, ^{13}C 90 atm %, Merck Sharp and Dohme, Montreal, Canada) in dry tetrahydrofuran was added to the mixture, the reaction mixture was heated under reflux for 2 hours. After cooling to room temperature it was poured into 100 ml of cold 1N H_2SO_4 and extracted with ether. The extract was washed with water and dried over sodium sulfate. The ether was evaporated and residue was purified by

a silica gel column chromatography using a mixture of benzene and ether as an eluent to give 6 (0.9 g, 75.0%). Mass 155 (M^+), 140; ^{1}H-NMR ($CDCl_3$) δ, 1.61 (d, 3H, J_{C-C-H}=5.0 Hz, $-CH_3$), 1.69 (d, 3H, J_{C-C-H}=5.0 Hz, $-CH_3$); ^{13}C-NMR ($CDCl_3$) δ, 131.4 (s, ^{13}C-enriched, =C-), 25.6 (dq, J_{C-C}=56.9 Hz, $-CH_3$), 17.6 (dq, J_{C-C}=57.9 Hz, $-CH_3$).

[12'-^{13}C]6-Methoxymethoxy-2,5,7,8-tetramethyl-2-[(3E,7E,9E)-5-mercaptothiazolinyl-4,8,12-trimethyl-3,7,9-tridecatrien-1-yl)-chroman (9). The mixture of [7-^{13}C]geraniol (130 mg, 0.8 mmol), carbon tetrabromide (332 mg, 1.0 mmol) and triphenylphosphine (236 mg, 1.0 mmol) in dry benzene was refluxed for 40 min. After cooling, the mixture was filtered and evaporated to give the crude [7-^{13}C]geranyl bromide (7) which was used in next step without purification. To a solution of 8 (134 mg, 0.3 mmol) in a mixture (25 ml) of tetrahydrofuran and hexamethylphosphoramide (24:1) being stirred under nitrogen gas in a dry ice-acetone bath was added n-butyl lithium (1.6 mol solution in hexane, 2 ml). After the stirring was continued for one hour, a solution of 7 (100 mg, 0.5 mmol) in tetrahydrofuran (5 ml) was added dropwise to the reaction mixture with stirring. After 2 hours, the reaction mixture was warmed to 0°C very slowly, poured into ice water and extracted with ethyl acetate. The organic layer was washed with water and dried over sodium sulfate. The ethyl acetate was evaporated and the residue was chromatographed on a silica gel column using benzene as an eluent. There was obtained 9 as a pale yellow oil (137 mg, 78.8%). IR (neat) 1570 cm^{-1}; UV (methanol) max 278 (ε:2600), 288 (ε:3400)nm; ^{1}H-NMR ($CDCl_3$) δ, 1.60 (d, 3H, J_{C-C-H}=5.0 Hz, $-CH_3$), 1.69 (d, 3H, J_{C-C-H}=5.0 Hz, $-CH_3$); ^{13}C-NMR ($CDCl_3$) δ, 131.2 (s, -C=, ^{13}C-enriched), 124.2 (dd, J_{C-C}=61.2 Hz, C=C-H), 26.6 (dq, J_{C-C}=43.5 Hz, $-CH_3$), 17.7 (dq, J_{C-C}=41.4 Hz, $-CH_3$).

[12'-^{13}C]6-Methoxymethoxy-2,5,7,8-tetramethyl-2-[(3E,7E,9E)-4,8,12-trimethyl-3,7,9-tridecatrien-1-yl]chroman (10). To a solution of 9 (130 mg) in tetrahydrofuran was added zinc powder (200 mg) and several drops of acetic acid. The mixture was stirred for 30 min at room temperature, then poured into ice water and extracted with ether. The extract was washed with a saturated sodium chloride solution, sodium bicarbonate solution and water, in turn. The ether was evaporated. The residue obtained was purified by a silica gel column chromatography using hexane as an eluent to give 10 (64.4 mg, 63.8%). Mass (M^+) 469; UV (methanol) max 276 (ε:2600), 289 (ε:3200)nm; ^{1}H-NMR ($CDCl_3$) δ, 1.60 (d, 3H, J_{C-C-H}=5.0 Hz, $-CH_3$), 1.68 (d, 3H, J_{C-C-H}=5.0 Hz, $-CH_3$); ^{13}C-NMR ($CDCl_3$) δ, 131.3 (s, =C-, ^{13}C-enriched), 25.6 (dq, J_{C-C}=42.6 Hz, $-CH_3$), 17.6 (dq, J_{C-C}=41.7 Hz, $-CH_3$).

[12'-^{13}C]*all-rac*-α-Tocopherol (1). The mixture of 10 (60 mg) and platinum oxide (120 mg) in ethyl acetate was shaken under 50 atm hydrogen at room temperature. After the theoretical amount of hydrogen was absorbed, the catalyst was removed by filteration. The filtrate was concentrated. The residue was dissolved into a hydrogen chloride-saturated methanol. After being stirred for 10 min at 0°C, the mixture was poured into ice water and extracted with ether. The ether solution was washed with water, a saturated sodium bicarbonate and water, in turn. After drying over sodium sulfate, the ether was evaporated. The residue was purified by a silica gel column chromatography using benzene as an eluent to yield 1 (55.1 mg, 89.5% from 9). Mass 431 (M^+), 416, 387; ^{1}H-NMR ($CDCl_3$) δ, 0.88 (dd, 6H, J_{C-C-H}=5.0 Hz, $-CH_3$); ^{13}C-NMR ($CDCl_3$) δ, 39.4 (dt, J_{C-C}=35.2 Hz,$-CH_2-$), 28.0 (d,-CH-, ^{13}C-enriched), 22.6 (dq, J_{C-C}=34.0 Hz, CH_3 x 2).

References and Notes

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