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The Synthesis of C-13 Labeled Vitamin E, [12′a,13′-^{13}C]*all-rac*-α-Tocopherol[1)]

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[12′a,13′-^{13}C]*all-rac*-α-Tocopherol (**1**) was synthesized from [1,3-^{13}C]acetone. The condensation of 6-methoxymethoxy-2,5,7,8-tetramethyl-2-[(*E*)-4-methyl-5-(thiazolin-2-yl)thio-3-penten-1-yl]chroman (**8**) with [7a,8-^{13}C]geranyl bromide (**7**), which was prepared by the coupling of (*E*)-(6-benzoyloxy-4-methyl-4-hexen-1-yl)triphenylphosphonium bromide (**5**) with [1,3-^{13}C]acetone, followed by bromination, afforded 6-methoxymethoxy-2,5,7,8-tetramethyl-2-[[12a,13-^{13}C](3*E*,7*E*,11*E*)4,8,12-trimethyl-5-(thiazolin-2-yl)thio-3,7,11-tridecatrien-1-yl]chroman (**9**). After desulfurization and reduction of **9**, the reaction product was converted to [12′a,13′-^{13}C]*all-rac*-α-tocopherol (**1**) by the use of methanolic hydrogen chloride. The overall yield of **1** was 33% on the basis of [1,3-^{13}C]acetone.

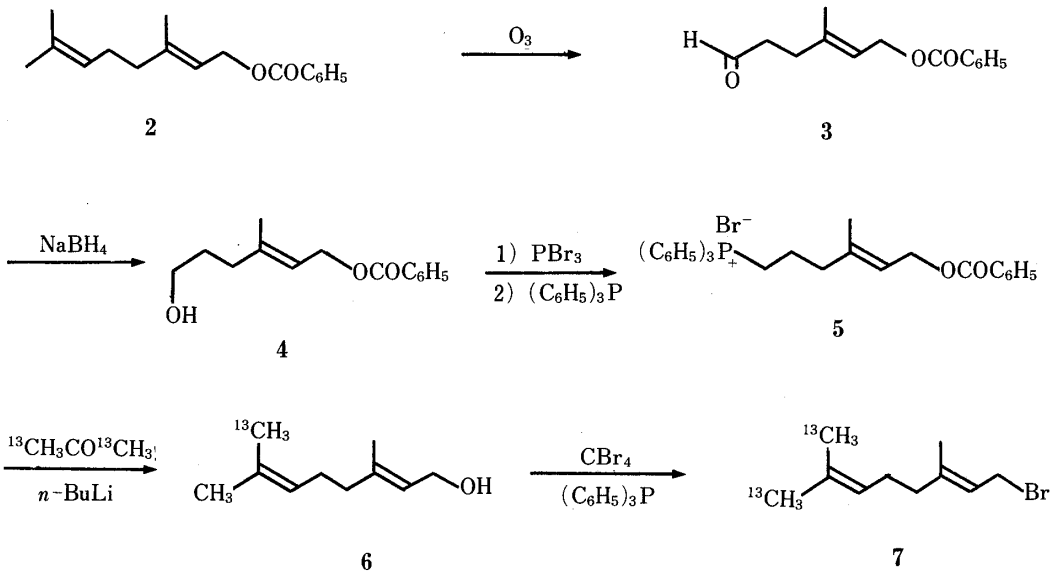
Keywords——vitamin E; synthesis; [12′a,13′-^{13}C]*all-rac*-α-tocopherol

Although vitamin E is presumed to be a biological antioxidant and radical scavenger, interest has recently been focused on interactions between its isoprenoid side chain and lipid components in biomembranes. Lucy *et al.*[2)] proposed, on the basis of studies with molecular models, that there are specific interactions between the isoprenoid side chain and fatty acyl chains of polyunsaturated phospholipids, particularly those derived from arachidonic acid. They suggested that this hypothesis could explain the membrane-stabilizing effect of α-tocopherol. One of the ways to assess the validity of this hypothesis is considered to be the measurement of the ^{13}C-relaxation time for α-tocopherol in biomembranes. α-Tocopherol having a ^{13}C-labeled isoprenoid moiety is necessary for such an investigation. We have recently established a new route for the synthesis of ^{13}C-labeled α-tocopherol.[3)] We now wish to report the preparation of [12′a,13′-^{13}C]*all-rac*-α-tocopherol (**1**).

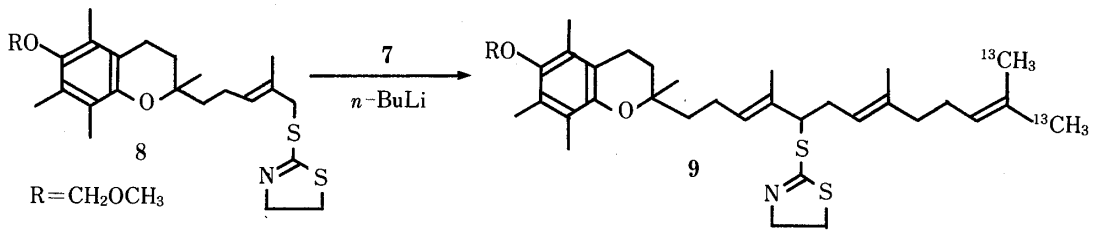
Geranyl benzoate (**2**) was ozonized in CH_2Cl_2 at -78 °C to give the aldehyde (**3**) in 94% yield. Although many previous reports on selective ozonolysis of geraniol derivatives, such as *O*-benzyl, *O*-THP and *O*-acetate derivatives, have appeared, only poor yields were obtained.[4)] Surprisingly, the use of geranyl benzoate afforded the aldehyde **3** in high yield. Compound **3** was reduced with $NaBH_4$ to give (*E*)-6-benzoyloxy-4-methyl-4-hexen-1-ol (**4**) in nearly quantitative yield. Then, compound **4** was treated with phosphorus tribromide to give the corresponding bromide, which was converted into the Wittig reagent, (*E*)-(6-benzoyloxy-4-methyl-4-hexen-1-yl)triphenylphosphonium bromide (**5**), in 89% overall yield. Condensation of **5** with [1,3-^{13}C]acetone in the presence of *n*-butyl-lithium affroded [7a,8-^{13}C]geraniol (**6**) in 75% yield. The labeled positions in **6** were confirmed by NMR spectroscopy; in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum ($CDCl_3$), the signals at 1.62 ppm and 1.70 ppm are split with the same coupling constant (both $J_{C\text{-}H}=126.0$ Hz) and in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum ($CDCl_3$) the intensities of the signals at 17.6 ppm and 35.6 ppm are greatly enhanced. The labeled geraniol **6** was converted into [7a,8-^{13}C]geranyl bromide (**7**) by the treatment with carbon tetrabromide and triphenylphosphine.[5)]

Because of its instability, **7** was used in the next step without purification.

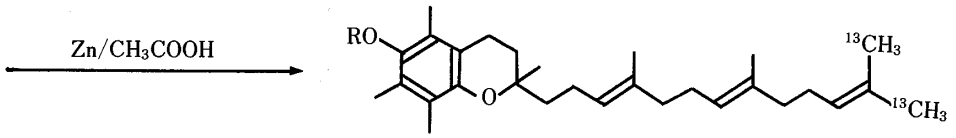
6-Methoxymethoxy-2,5,7,8-tetramethyl-2-[(*E*)-4-methyl-5-(thiazolin-2-yl)thio-3-penten-1-yl]chroman (**8**)[3a] was lithiated with *n*-butyl-lithium, and then treated with **7** to give the coupling product, 6-methoxymethoxy-2,5,7,8-tetramethyl-2-[[12a,13-^{13}C](3*E*,7*E*,11*E*)-4,8,12-trimethyl-5-(thiazolin-2-yl)thio-3,7,11-tridecatrien-1-yl]chroman (**9**), in 78% yield.



2 **3** **4** **5** **6** **7**



8 **9**



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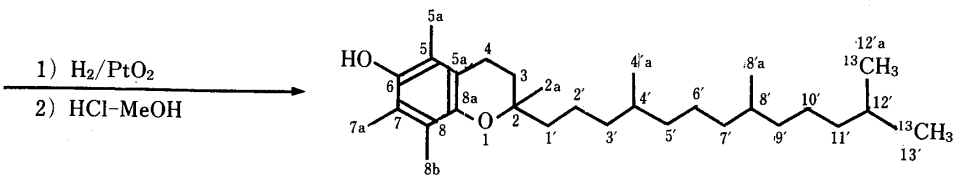

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Desulfurization of **9** with zinc powder led to 6-methoxymethoxy-2,5,7,8-tetramethyl-2-[[12a,13-^{13}C](3*E*, 7*E*, 11*E*)4,8,12-trimethyl-3,7,11-tridecatrien-1-yl]chroman (**10**) in 63% yield. Finally, **10** was reduced under 50 atm of hydrogen in the presence of platinum oxide, and the methoxymethyl protecting group was removed by the use of methanolic hydrogen chloride. The desired [12′a,13′-^{13}C]*all-rac*-α-tocopherol (**1**) was obtained in 89% yield from **10**. The ^{13}C-labeled positions were confirmed on the basis of the marked enhancement of the signal at 22.6 ppm (C-12′a and C-13′) and the coupling between the labeled methyl carbons and C-12′ ($J_{C\text{-}C}$ = 38.9 Hz) in the ^{13}C-NMR spectrum. The overall yield of **1** based on [1,3-^{13}C]acetone was 33%.

Experimental

Mass (MS), ultraviolet (UV) and infrared (IR) spectra were taken with Shimadzu-LKB 9000, Cary 118C and Jasco IRA-2 spectrometers, respectively. Gas chromatograms were obtained on a Shimadzu GC-5A instrument with a flame ionization detector. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian XL-200 spectrometer employing tetramethylsilane as an internal standard. Silica gel C-200 (Wako, Osaka, Japan) was used for column chromatography and Silica gel PF_{254} (Merck, Darmstadt, BRD) for thin layer chromatography. [1,3-^{13}C]acetone (^{13}C 90 atom percent) was purchased from Merck Sharp and Dohme Limited (Montreal, Canada).

(*E*)-6-Benzoyloxy-4-methyl-2-hexenal (3)——A stream of ozone in oxygen (flow rate, 2.2 mmol/min) generated with an ozonator was bubbled through a solution of geranyl benzoate (10.0 g, 38.8 mmol) in methylene chloride in a dry ice–acetone bath for 4 h. The reaction mixture was warmed to room temperature, washed with water, and dried over sodium sulfate. The methylene chloride solution was concentrated. The residue was purified by silica gel column chromatography using benzene as an eluent to give **3** as a colorless oil (8.5 g, 94%). MS *m/e*: 232. IR (neat): 1685, 1750 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 9.78 (br t, 1H, C$\underline{H}$O), 4.86 (d, 2H, J = 7.5 Hz, C$\underline{H}_2$–O). ^{13}C-NMR ($CDCl_3$) δ: 201.5 (d, $\underline{C}$HO), 61.6 (5, $\underline{C}H_2$–O), 16.6 (q, $\underline{C}H_3$). *Anal.* Calcd for $C_{14}H_{16}O_3$: C, 72.41; H, 6.90. Found: C, 72.71; H, 6.77.

(*E*)-6-Benzoyloxy-4-methyl-2-hexen-1-ol (4)——Sodium borohydride (1.0 g, 26 mmol) was added to a solution of **3** (6.1 g, 26 mmol) in methanol (100 ml) with stirring at 0 °C. The stirring was continued for 3 h. The reaction mixture was poured into ice water (150 ml) and then extracted with ether. The ether extract was washed with 10% acetic acid and water successively. The extract was dried and concentrated. The residue was applied to a silica gel column and eluted with a mixture of benzene and ether (5 : 1) to yield **4** as an oil (6.1 g, 99%). MS *m/e*: 234. IR (neat): 3390, 1751 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.87 (d, 2H, J = 7.5 Hz, C$\underline{H}_2$–O), 3.66 (t, 2H, J = 7.5 Hz, C$\underline{H}_2$–OH), 1.78 (s, 3H, C$\underline{H}_3$). ^{13}C-NMR ($CDCl_3$) δ: 63.1 (t, $\underline{C}H_2$–O), 61.8 (t, $\underline{C}H_2$–OH). *Anal.* Calcd for $C_{14}H_{18}O_3$: C, 71.79; H, 7.69. Found: C, 71.81; H, 7.78.

(*E*)-6-Benzoyloxy-4-methyl-4-hexen-1-yl)triphenylphosphonium Bromide (5)——Phosphorus tribromide (4.6 g) was added with stirring at 0 °C to a solution of **4** (4.0 g, 17.0 mmol) in dry chloroform (100 ml). The stirring was continued for 30 min. The mixture was poured into ice water and extracted with chloroform. The chloroform solution was washed with water and then dried over sodium sulfate. The chloroform solution was concentrated to give a crude bromide (4.5 g). A solution of the product in dry toluene (30 ml) was added, with stirring under reflux, to a solution of triphenylphosphine (3.7 g, 14.0 mmol) in dry toluene (30 ml), and refluxing was continued for 15 h. The solvent was evaporated at 80 °C. The residue was triturated with cold toluene and filtered. The white solid thus obtained was dried in a vacuum oven at 50 °C to yield **5** (7.6 g, 89%). IR (neat): 1750 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 4.86 (d, 2H, J = 7.5 Hz, C$\underline{H}_2$–O), 3.24 (m, 2H, $J_{P\text{-}C\text{-}H}$ = 12.0 Hz, P–C$\underline{H}_2$), 1.68 (s, 3H, C$\underline{H}_3$). ^{13}C-NMR (CD_3OD) δ: 40.4 (dt, $J_{C\text{-}C\text{-}C\text{-}P}$ = 16.1 Hz, $\underline{C}H_2$), 21.9 (dt, $J_{C\text{-}P}$ = 52.2 Hz, $\underline{C}H_2$), 21.1 (dt, $J_{C\text{-}C\text{-}P}$ = 3.6 Hz, $\underline{C}H_2$). *Anal.* Calcd for $C_{32}H_{32}BrO_2P$: C, 68.82; H, 5.73; O, 5.73. Found: C, 69.01; H, 5.50; O, 5.71.

[7a,8-^{13}C]Geraniol (6)——A solution of *n*-butyllithium (10 ml, 1.6 mol) in dry tetrahydrofuran (THF) was added with stirring at 0 °C under nitrogen to a suspension of **5** (4.5 g, 8.0 mmol) in dry THF (50 ml). The stirring was continued for 30 min at room temperature, then a solution of [1,3-^{13}C]acetone (0.5 g) in dry THF was added, and the reaction mixture was heated under reflux for 2 h. After being cooled to room temperature, it was poured into 100 ml of cold 1 N H_2SO_4 and extracted with ether. The ether was evaporated. The residue was purified by silica gel chromatography using a mixture of benzene and ether (5 : 1) as an eluent to give **6** as a colorless oil (0.9 g, 75%). MS *m/e*: 156.1427 (M^+) (Calcd 156.1423). ^{1}H-NMR ($CDCl_3$) δ: 1.62 (dd, 3H, $J_{C\text{-}H}$ = 126.0 Hz, $J_{C\text{-}C\text{-}C\text{-}H}$ = 4.0 Hz, C$\underline{H}_3$), 1.70 (dd, 3H, $J_{C\text{-}H}$ = 126.0 Hz, $J_{C\text{-}C\text{-}C\text{-}H}$ = 4.0 Hz, C$\underline{H}_3$). ^{13}C-NMR ($CDCl_3$) δ: 17.6 (q, $\underline{C}H_3$, ^{13}C-enriched), 35.6 (q, $\underline{C}H_3$, ^{13}C-enriched).

6-Methoxymethoxy-2,5,7,8-tetramethyl-2-[[12a,13-^{13}C](3*E*, 7*E*, 11*E*)-4,8,12-trimethyl-5-(thiazolin-2-yl)thio-3,7,11-tridecatrien-1-yl]chroman (9)——A mixture of [7a,8-^{13}C]geraniol (0.13 g, 0.8 mmol), carbon tetrabromide (0.33 g, 1.0 mmol) and triphenylphosphine (0.24 g, 1.0 mmol) in dry benzene was refluxed for 40 min. After being cooled, the mixture was filtered and concentrated to give crude [7a,8-^{13}C]geranyl bromide (**7**), which was used in the

next step without purification. A solution of *n*-butyllithium in hexane (1.6 mol, 2 ml) was added to a solution of **8** (0.134 g, 0.3 mmol) in a mixture (25 ml) of THF and hexamethylphosphoramide (24 : 1) with stirring under nitrogen in a dry ice–acetone bath. The stirring was continued for 1 h, then a solution of **7** (0.1 g, 0.5 mmol) in THF (5 ml) was added. After 3 h, 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. The residue was applied to a silica gel column and eluted with benzene to yield **9** as a pale yellow oil (0.137 g, 78%). MS *m/e*: 587.3374 (M^+), (Calcd 587.3377). IR (neat): 1570 cm^{-1}. UV $\lambda_{max}^{methanol}$ nm (ε): 278 (2600), 288 (3400). ^{1}H-NMR ($CDCl_3$) δ: 1.60 (dd, 3H, $J_{C\text{-}H}$=126.0 Hz, $J_{C\text{-}C\text{-}C\text{-}H}$=4.0 Hz, $C\underline{H}_3$), 1.69 (dd, 3H, $J_{C\text{-}H}$=126.0 Hz, $J_{C\text{-}C\text{-}C\text{-}H}$=4.0 Hz, $C\underline{H}_3$). ^{13}C-NMR ($CDCl_3$) δ: 131.2 (dd, $J_{C\text{-}C}$=40.3 Hz, $J_{C\text{-}C}$=41.2 Hz, $\underline{C}$=C), 26.6 (q, $\underline{C}H_3$, ^{13}C-enriched), 17.7 (q, $\underline{C}H_3$, ^{13}C-enriched).

6-Methoxymethoxy-2,5,7,8-tetramethyl-2-[[12a,13-^{13}C](3*E*, 7*E*, 11*E*)-4,8,12-trimethyl-3,7,11-tridecatrien-1-yl]chroman (10)——Zinc powder (0.2 g) and several drops of acetic acid were added to a solution of **9** (0.13 g) in THF. The mixture was stirred for 30 min at room temperature, poured into ice–water and extracted with ether. The extract was washed with water, saturated sodium bicarbonate solution and water successively. The ether was evaporated. The residue was purified by silica gel column chromatography using hexane as an eluent to yield **10** as an oil (0.064 g, 63%). MS *m/e*: 470.3667 (M^+), (Calcd 470.3661). UV $\lambda_{max}^{methanol}$ nm (ε): 276 (2600), 289 (3200). ^{1}H-NMR ($CDCl_3$) δ: 1.60 (dd, 3H, $J_{C\text{-}H}$=126.0 Hz, $J_{C\text{-}C\text{-}C\text{-}H}$=4.0 Hz, $\underline{C}H_3$), 1.68 (dd, 3H, $J_{C\text{-}H}$=126.0 Hz, $\underline{C}H_3$). ^{13}C-NMR ($CDCl_3$) δ: 131.3 (dd, $J_{C\text{-}C}$=40.2 Hz, $J_{C\text{-}C}$=42.5 Hz, $\underline{C}$=C), 25.6 (q, $\underline{C}H_3$, ^{13}C-enriched), 17.6 (q, $\underline{C}H_3$, ^{13}C-enriched).

[12′a,13′-^{13}C]*all-rac*-α-Tocopherol (1)——A mixture of **10** (0.06 g) and platinum oxide (0.12 g) in ethyl acetate was shaken under 50 atm of hydrogen at room temperature. After the calculated amount of hydrogen had been consumed, the catalyst was filtered off. The filtrate was concentrated. The residue was dissolved in 10% HCl–MeOH (2 ml) and the solution was stirred at room temperature for 10 min, then concentrated to yield **1** as a colorless oil (0.55 g, 89% from **10**). MS *m/e*: 432.3875 (M^+), (Calcd for 432.3870). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (dd, 6H, $J_{C\text{-}H}$=126.0 Hz, $J_{H\text{-}H}$=7.5 Hz, $C\underline{H}_3$). ^{13}C-NMR ($CDCl_3$) δ: 28.0 (dd, $J_{C\text{-}C}$=38.9 Hz, $\underline{C}H$), 22.6 (q, $\underline{C}H_3$, ^{13}C-enriched).

References and Notes

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