Preparation of (26-^{13}C)Desmosterol

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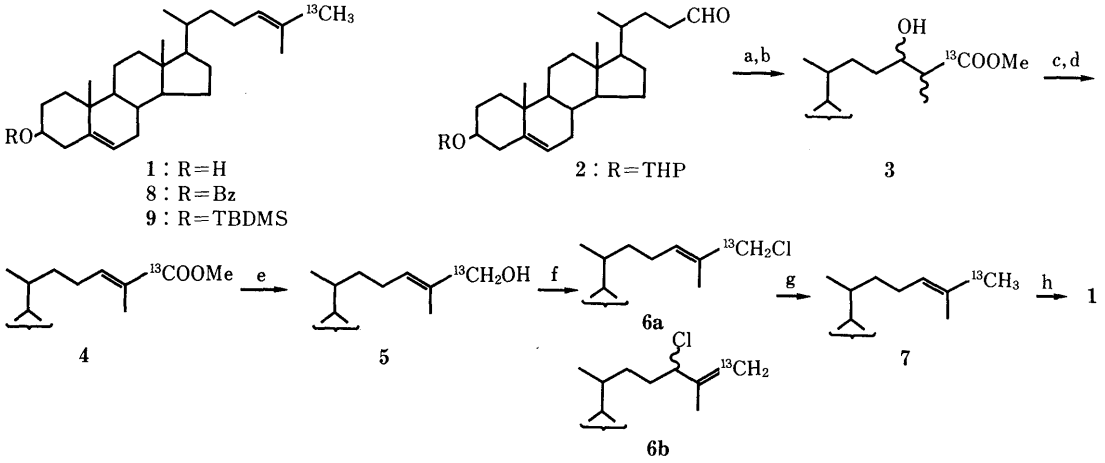
(26-^{13}C)Desmosterol (1) and its 3-benzoate (8) and 3-*tert*-butyldimethylsilyl ether (9) were synthesized starting with (1-^{13}C)propionic acid and a steroidal C-24 aldehyde (2).

Keywords (26-^{13}C)desmosterol; desmosterol; carbon-13 label; (1-^{13}C)propionic acid; ^{13}C-NMR; prochirality

Sterols have a prochiral center at the C-25 position and the C-26 and C-27 methyl groups are diastereotopic. In general, enzymes associated with the transformation of the isopropyl group recognize this prochirality. For example, it has been established that the *pro-R* (C-26) methyl of cholesterol is stereoselectively oxidized in the formation of bile acid[1] and that the *pro-R* methyl of cholesterol originates from C-2 of mevalonate in cholesterol biosynthesis in rats.[2] The stereochemical mode in the formation of phytosterol and ergosterol from a 24(25)-olefinic precursor, whereby the cryptic stereochemistry at C-25 is determined, has been recently reported.[3] Our continuing interest in the mechanisms of sterol metabolism and of chemical reactions prompted us to examine the fate of the diastereotopic C-26 and C-27 groups. For this purpose, we required desmosterol stereospecifically labeled at either C-26 or C-27 as a key intermediate. Labeling with ^{13}C seems to be appropriate since the metabolic fate of the methyl group can be followed conveniently by ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectroscopy. Thus, (26-^{13}C)desmosterol (**1**)[4] was chosen as our synthetic target. Previously reported syntheses of non-labeled desmosterol[5] were not suitable for stereoselective introduction of ^{13}C at the C-26 position of **1**. Preparation of [26-^{3}H]desmosterol has been reported.[6] In this paper, we described the stereoselective synthesis of **1**.

Chart 1 illustrates our synthetic route. (1-^{13}C)Propionic acid (99 atom % enriched) was used as a starting ^{13}C source. The coupling reaction of Li dianion, generated from (1-^{13}C)propionic acid,[7] with 3β-(tetrahydropyranyloxy)-chol-5-en-24-al (**2**)[8] in tetrahydrofuran (THF) at room temperature afforded a mixture of diastereoisomeric hydroxy acids. Diazomethane treatment of the acids gave the corresponding methyl ester **3** (78% based on the ^{13}C-propionic acid). The contents of the four diastereoisomers (due to the C-24 and C-25 chiral centers) were found to be approximately equal based on the ^{13}C-NMR spectrum of **3** (also that of the mesylate). The ester **3** was converted into the intermediary mesylate, which in turn was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at reflux in benzene to afford the unsaturated ester (4) in 93% yield from **3**. The ^{13}C-NMR spectrum of **4** showed only one signal ascribable to a carboxyl group, thus indicating that **4** is exclusively the (*E*)-isomer. Reduction of **4** with diisobutylaluminum hydride (DIBAL) gave the allylic alcohol **5** in 92% yield. The ^{1}H-NMR spectrum of **5** exhibited an oxymethylene signal (26-H_2) at δ 3.99 as a doublet with $^{1}J_{C-H}$ = 142 Hz and a methyl signal (27-H_3) at δ 1.67 as a doublet with $^{3}J_{C-H}$ = 4.2 Hz. Since the ^{13}C-NMR spectrum of **5** showed two peaks at δ 69.07 and 61.66 in a 21 : 1 intensity ratio, it was indicated that *ca.* 5% of (*Z*)-isomer was contained in **5**. Treatment of **5** with thionyl chloride–pyridine afforded a mixture of the C-26 chloride **6a** and the C-24 chloride **6b** in a 2 : 1 ratio, as indicated by ^{1}H-NMR. The mixture was reduced with $LiAlH_4$ at reflux in ether to afford desmosterol THP ether (**7**; THP = tetrahydropyranyl) in 51% yield from **5**. The secondary chloride **6b** was not completely reduced under these conditions and was partially recovered. Deprotection of **7** under acidic conditions furnished (26-^{13}C)desmosterol (**1**).



a) $CH_3CHLi^{13}COOLi$ b) CH_2N_2 c) CH_3SO_2Cl-Et_3N-DMAP d) DBU e) $(iso\text{-}Bu)_2AlH$ f) $SOCl_2$-Py g) $LiAlH_4$ h) HCl

Chart 1

The ^{13}C-NMR spectrum of **1** indicated that the ^{13}C label is located 92% at C-26 (δ 25.73) and 8% at C-27 (δ 17.65). The mass spectrum (MS) of **1** exhibited a molecular ion at *m/z* 385, whereas an ion of *m/z* 384 was not observed within the limit of detection. The ^{13}C-desmosterol thus obtained was converted into the benzoate **8** and *tert*-butyldimethylsilyl (TBDMS) ether **9** in a standard manner for further transformations.

The ether **9** was manipulated to (26-^{13}C)- and (27-^{13}C)-fucosterol 24,28-epoxides according to the published method,[9] and the epoxides have been employed in our recent work on the stereochemical fate of the diastereotopic methyl groups during the conversion of fucosterol epoxide into desmosterol in insects.[10] (24*R*,25*R*)- and (24*S*,25*S*)-Desmosterol 24,25-epoxides specifically labeled at one of the diastereotopic methyl groups (C-26), easily obtained from the benzoate **8**, have been a key substrate in the study on the mechanism of Lewis acid-catalyzed epoxide-ketone rearrangement.[11]

Experimental

General Melting points were determined on a hot-stage microscope and are uncorrected. ^1H- and ^{13}C-NMR spectra were recorded on a JEOL FX-200 or JEOL GSX-500 spectrometer in $CDCl_3$ with tetramethylsilane as an internal reference. MS (70 eV) were recorded on a Shimadzu GC-MS DF 9020 spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck, 70—230 mesh). Extractive work-up refers to dilution of a reaction mixture with water (or the indicated solution), extraction with the given organic solvent, washings of the extract to neutrality, drying over Na_2SO_4, filtration, and removal of the solvent under reduced pressure. Parallel experiments were carried out with non-labeled materials and analytical samples were prepared for non-labeled samples in most cases in order to economize on the use of labeled materials.

(26-^{13}C)-24-Hydroxy-3β-(tetrahydropyranyloxy)cholest-5-en-26-oic Acid Methyl Ester (Diastereoisomeric Mixture) (3) *n*-Butyl lithium (1.59 M hexane solution, 2.77 ml, 4.40 mmol) was added to a solution of diisopropylamine in dry THF (1.6 ml) at 0 °C under nitrogen and the mixture was stirred for 0.5 h at the same temperature. (1-^{13}C)Propionic acid (151 μl, 2.00 mmol) was added to the solution at −60 °C and the whole mixture was stirred at 25 °C for 1 h. The solvent was removed under reduced pressure, then dry THF (3.0 ml) was added and the mixture was finally cooled to −60 °C. The aldehyde **2** (1.06 g, 2.40 mmol) in dry THF (2.0 ml) was added to the solution with stirring, which was continued for 1 h at the same temperature. Then, saturated aqueous NH_4Cl, chloroform, and finally 6 N HCl (until the medium became acidic) were added to the reaction mixture. The chloroform layer was separated, dried and concentrated to dryness. The residue dissolved in THF was treated with excess ethereal diazomethane at room temperature for 1 h. Evaporation of the solvent afforded a crude product, which was chromatographed on silica gel. Elution with hexane–ethyl acetate (6:1) gave the methyl ester **3** (830 mg, 78% based on ^{13}C-propionic acid) as a solid. ^1H-NMR δ: 0.68 (3H, s, 18-H_3), 0.92 (3H, d, J=6.4 Hz, 21-H_3), 1.01 (3H, s, 19-H_3), 3.4—3.7 (3H, m, 3-H, 24-H, 6′-H of THP), 3.71 (3H, d, $^3J_{C-H}$=3.7 Hz, COOMe), 3.8—4.0 (1H, m, 6′-H of THP), 4.70 (1H, m, 2′-H of THP), 5.35 (1H, m, 6-H). ^{13}C-NMR, signals due to COOMe of the four isomers were observed at δ 176.37, 176.37, 176.45, 176.57. Non-labeled sample, mp 86.5—90 °C (from hexane–ethyl acetate). ^1H-NMR δ: 3.71 (3H, s, COOMe). *Anal.* Calcd for $C_{33}H_{54}O_5$: C, 74.72; H, 10.19. Found: C, 74.85; H, 10.28.

(26-^{13}C)-3β-(Tetrahydropyranyloxy)cholesta-5,24-dien-26-oic Acid Methyl Ester (4) A mixture of **3** (807 mg, 1.52 mmol), methanesulfonyl chloride (235 μl, 3.04 mmol), triethylamine (846 μl, 6.07 mmol), and 4-dimethylaminopyridine (DMAP) (catalytic amount) in dry methylene chloride (8.0 ml) was stirred at 0 °C for 50 min. Ice chips were added to the mixture, and extractive (ether) work up gave a crude product, which was chromatographed on silica gel. Elution with hexane–ethyl acetate (6:1) gave the mesylate (965 mg, 104%). ^1H-NMR δ: 0.67 (3H, s, 18-H_3), 0.94 (3H, d, J=5.6 Hz, 21-H_3), 1.01 (3H, s, 19-H_3), 3.003, 3.003, 3.008, 3.015 (3H, each s, SO_2Me), 3.4—3.6 (2H, m, 3-H, 6′-H of THP), 3.72, 3.73 (3H, each d, $^3J_{C-H}$=3.9 Hz, COOMe), 3.8—4.0 (1H, m, 6′-H of THP), 4.70 (1H, m, 2′-H of THP), 4.94 (1H, m, 24-H), 5.35 (1H, m, 6-H). ^{13}C-NMR, signals due to COOMe of the four isomers were observed at δ 173.35, 173.40, 173.48, 173.48. Non-labeled sample, mp 90—99.5 °C (from hexane–ethyl acetate). ^1H-NMR δ: 3.001, 3.001, 3.007, 3.014 (3H, each s, SO_2Me), 3.71, 3.73 (3H, each s, COOMe).

1,8-Diazabicyclo[5.4.0]undec-7-ene (454 μl, 3.04 mmol) was added to a stirred solution of the mesylate (965 mg, 1.52 mmol) in dry benzene (40 ml) at room temperature. The mixture was heated at 75 °C for 7 h. Extractive (ether) work-up gave a crude product, which was chromatographed on silica gel. Elution with hexane–ethyl acetate (10:1) afford the unsaturated **4** (723 mg, 93% from **3**). ^1H-NMR δ: 0.68 (3H, s, 18-H_3), 0.96 (3H, d, J=6.6 Hz, 21-H_3), 1.01 (3H, s, 19-H_3), 1.83 (3H, d, $^3J_{C-H}$=2.9 Hz, 27-H_3), 3.4—3.6 (2H, m, 3-H, 6′-H of THP), 3.73 (3H, d, $^3J_{C-H}$=3.7 Hz, COOMe), 3.8—4.0 (1H, m, 6′-H of THP), 4.7 (1H, m, 2′-H of THP), 5.35 (1H, m, 6-H), 6.76 (1H, q, J_{H-H}=$^3J_{C-H}$=6.8 Hz, 24-H). ^{13}C-NMR δ: 168.7 (C-26). Non-labeled sample, mp 109—113 °C (from methanol). ^1H-NMR δ: 1.84 (3H, s, 26-H_3), 3.73 (3H, s, COOMe), 6.75 (1H, t, J=6.8 Hz, 24-H). *Anal.* Calcd for $C_{33}H_{52}O_4$: C, 77.34; H, 10.16. Found: C, 77.32; H, 10.21.

(26-^{13}C)-26-Hydroxy-3β-(tetrahydropyranyloxy)cholesta-5,24-diene (5) Diisobutylaluminum hydride (1.75 M hexane solution, 1.9 ml, 3.36 mmol) was added dropwise to a stirred solution of **4** (695 mg, 1.35 mmol) in dry ether (10 ml) at room temperature. After 50 min, the reaction was terminated by addition of water. Extractive (chloroform) work-up gave a crude product, which was chromatographed on silica gel. Elution with hexane–ethyl acetate (6:1) afforded the allylic alcohol **5** (602 mg, 92%), mp 116—117.5 °C (from hexane–ethyl acetate). ^1H-NMR δ: 0.68 (3H, s, 18-H_3), 0.95 (3H, d, J=6.6 Hz, 21-H_3), 1.01 (3H, s, 19-H_3), 1.67 (3H, d, $^3J_{C-H}$=4.2 Hz, 27-H_3), 3.4—3.6 (2H, m, 3-H, 6′-H of THP), 3.8—4.0 (1H, m, 6′-H of THP), 3.99 (2H, d, $^1J_{C-H}$=142 Hz, 26-H_2), 4.7 (1H, m, 2′-H of THP), 5.3—5.5 (2H, m, 6-H, 24-H). ^{13}C-NMR showed two signals: δ 69.07 and 61.66 (CH_2OH) with a 21:1 intensity. Non-labeled sample, ^1H-NMR δ: 1.67 (3H, s, 27-H_3), 4.00 (2H, s, 26-H_2). *Anal.* Calcd for $C_{32}H_{52}O_3$: C, 79.34; H, 10.74. Found: C, 79.29; H, 10.74.

(26-^{13}C)Desmosterol 3-Tetrahydropyranyl Ether (7) A mixture of **5** (302 mg, 0.62 mmol), thionyl chloride (90 μl, 1.23 mmol), and pyridine (110 μl, 1.36 mmol) in dry THF (3.0 ml) was stirred for 5 min at −12 °C and then for 30 min at room temperature. Extractive (ether) work-up gave a solid mixture (278 mg) of the 26-chloride **6a** and the 24-chloride **6b**. The ^1H-NMR spectrum of the mixture showed signals due to the isomeric chlorides: **6a** (2/3 part), 1.73 (3H, d, $^3J_{C-H}$=4.4 Hz, 27-H_3), 4.02 (2H, d, $^1J_{C-H}$=150 Hz, 26-H_2), 5.50 (1H, q, J_{H-H}=$^3J_{C-H}$=7.6 Hz, 24-H); **6b** (1/3 part), 1.79 (3H, d, $^3J_{C-H}$=6.4 Hz, olefinic methyl), 4.33 (1H, m, 24-H), 4.88 (1H, d, $^1J_{C-H}$=158 Hz, exomethylene-Ha), 4.99 (1H, d, $^1J_{C-H}$=158 Hz, exomethylene-Hb).

Lithium aluminum hydride (90 mg, 2.37 mmol) was added to a stirred solution of the chloride (278 mg) in dry ether (10 ml). The mixture was refluxed for 7 h and diluted with moist ether. Extractive (ether) work-up afforded a crude product, which was separated by flash chromatography on silica gel (Merck, Kieselgel 60, 230—400 mesh). Elution with hexane–ether (10:1) gave a partially purified **7**. Further purification by preparative thin layer chromatography (Merck, 20×20 cm, 0.5 mm thickness, Kieselgel 60 F_{254} precoated plates, developed three times with hexane–ether 10:1). Compound **7** (149 mg, 51%) was obtained from the more mobile band (*Rf* 0.54), mp 114.5—120 °C (hexane–ethyl acetate). ^1H-NMR δ: 0.68 (3H, s, 18-H_3), 0.93 (3H, d, J=6.4 Hz, 21-H_3), 1.01 (3H, s, 19-H_3), 1.60 (3H, d, $^3J_{C-H}$=4.2 Hz, 27-H_3), 1.68 (3H, d, $^1J_{C-H}$=125 Hz, 26-H_3), 3.4—3.65 (2H, m, 3-H, 6′-H of THP), 3.8—4.0 (1H, m, 6′-H of THP), 4.7 (1H, m, 2′-H of THP), 5.1 (1H, q, J_{H-H}=$^3J_{C-H}$=6.8 Hz, 24-H). ^{13}C-NMR δ: 25.73 (C-26) and 17.65 (C-27) with a 11:1 intensity ratio. Non-labeled sample, mp 121—122.5 °C (from acetone). ^1H-NMR δ: 1.60 (3H, s, 27-H_3), 1.68 (3H, s, 26-H_3), 5.06 (1H, t, J=6.8 Hz, 24-H). *Anal.* Calcd for $C_{32}H_{52}O_2$: C, 82.05; H, 11.11. Found: C, 82.13; H, 11.05.

The less mobile band (*Rf* 0.49) afforded the 24-chloride **6b** (30 mg, 10%), mp 123—130.5 °C (from acetone). The ^1H-NMR spectrum was as described above. ^{13}C-NMR δ: 113.87, 114.16 (exomethylene carbons, due to C-24 epimers). *Anal.* Calcd for C_{31} (^{13}C)$H_{51}ClO_2$: C, 76.43; H, 10.20. Found: C, 76.19; H, 10.26.

(26-^{13}C)Desmosterol (1) A mixture of **7** (200 mg, 0.43 mmol) and 2 N HCl (100 μl) in THF (1.5 ml) and methanol (1.5 ml) was stirred at room temperature for 2 h. Extractive (ether) work-up gave a crude product, which was chromatographed on silica gel. Elution with hexane–ethyl acetate (6:1) afforded **1** (138 mg, 84%), mp 116—118 °C (from methanol). ^1H-NMR δ: 0.68 (3H, s, 18-H_3), 0.94 (3H, d, J=6.4 Hz, 21-H_3), 1.01 (3H, s, 19-H_3), 1.60 (3H, d, $^3J_{C-H}$=3.9 Hz, 27-H_3), 1.68 (3H, d, $^1J_{C-H}$=125 Hz, 26-H_3), 3.5 (1H, m, 3-H), 5.1 (1H, q, J_{H-H}=$^3J_{C-H}$=6.8 Hz, 24-H), 5.35

(1H, m, 6-H). ^{13}C-NMR δ: 25.76 (C-26) and 17.68 (C-27) with a 11:1 intensity ratio. Non-labeled sample, mp 119—120.5 °C (from methanol) (lit. 118—120 °C,[12] 120—122 °C[13]). ^{1}H-NMR δ: 1.60 (3H, s, 27-H_3), 1.68 (3H, s, 26-H_3), 5.1 (1H, t, J=6.8 Hz, 24-H). *Anal.* Calcd for $C_{27}H_{44}O$: C, 84.38; H, 11.46. Found: C, 84.12; H, 11.43.

(26-^{13}C)Desmosterol · Benzoate (8) A mixture of **1** (20 mg, 0.052 mmol), benzoyl chloride (18 μl, 0.156 mmol) and 4-dimethyl-aminopyridine (catalytic amount) in pyridine (1.0 ml) was stirred at room temperature for 10 h. Extractive (ethyl acetate) work-up gave a crude product, which was chromatographed on silical gel. Elution with hexane–ethyl acetate (20:1) afforded **2** (25 mg, 98%), mp 130—132 °C (from acetone). ^{1}H-NMR δ: 0.69 (3H, s, 18-H_3), 0.94 (3H, d, J=6.6 Hz, 21-H_3), 1.07 (3H, s, 19-H_3), 1.61 (3H, d, $^3J_{C-H}$=3.6 Hz, 27-H_3), 1.69 (3H, d, $^1J_{C-H}$=125 Hz, 26-H_3), 4.86 (1H, m, 24-H), 5.09 (1H, q, J_{H-H}=$^3J_{C-H}$=7.2 Hz, 24-H), 5.42 (1H, m, 6-H), 7.4—7.6, 8.0—8.1 (5H, m, aromatic H_5), ^{13}C-NMR δ: 25.75 (C-26), 17.67 (C-27) with a 11:1 intensity ratio. Non-labeled sample, mp 134—135 °C (from acetone) (lit. 131 °C).[14]

(26-^{13}C)Desmosterol *tert*-Butyldimethylsilyl Ether (9) A mixture of **1** (154 mg, 4.0 mmol), imidazole (82 mg, 12 mmol), *tert*-butyldimethylsilyl chloride (90 mg, 6.0 mmol), 4-dimethylaminopyridine (catalytic amount) in dimethylformamide (3.0 ml) was stirred at room temperature for 13 h. Extractive (ether) work-up gave a crude product, which was chromatographed on silica gel. Elution with hexane–ether (10:1) afforded **9** (200 mg, 100%), mp 123—125 °C (from hexane–ethyl acetate). ^{1}H-NMR δ: 0.06 (6H, s, $SiMe_2$), 0.67 (3H, s, 18-H_3), 0.89 (9H, s, *tert*-Bu), 0.93 (3H, d, J=6.6 Hz, 21-H_3), 1.00 (3H, s, 19-H_3), 1.60 (3H, d, $^3J_{C-H}$=3.7 Hz, 27-H_3), 1.68 (3H, d, $^1J_{C-H}$=125 Hz, 26-H_3), 3.48 (1H, m, 3-H), 5.1 (1H, q, J_{H-H}=$^3J_{C-H}$=6.8 Hz, 24-H). ^{13}C-NMR δ: 25.76 and 17.68 with a 11:1 intensity ratio. Non-labeled sample, mp 127.0—127.5 °C (from acetone). ^{1}H-NMR δ: 1.60 (3H, s, 27-H_3), 1.68 (3H, s, 26-H_3), 5.08 (1H, t, J=6.8 Hz, 24-H). *Anal.* Calcd for $C_{33}H_{58}OSi$: C, 79.52; H, 11.65. Found: C, 79.33; H, 11.80.

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