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Convenient Preparation of the C-24 Stereoisomers of 24-Ethyl- and 24-Methylcholesterols[1]

Yoshinori Fujimoto and Nobuo Ikekawa*

Laboratory of Chemistry for Natural Products, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama, 227, Japan

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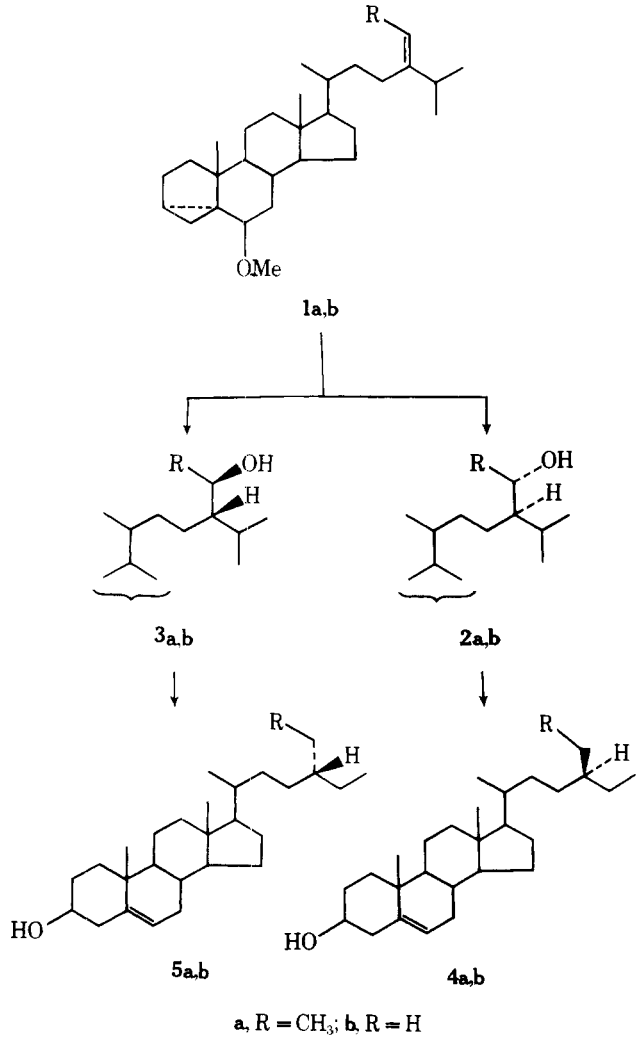
Although syntheses of (24*R*)- and (24*S*)-alkylsterols have already been reported,[2,3] we have searched for another simple method of their preparation. The one described here allows the introduction of tritium or deuterium stereospecifically at C-24 and/or C-28 of 24-alkylsterols and may be useful for the stereochemical investigations of C-24(28) dehydrogenation, which is the first step of sterol metabolism in phytophagous insects.[4]



Hydroboration–oxidation of the 3,5-cyclo derivative (**1a**) of fucosterol gave a diastereoisomeric mixture of 28-hydroxyl derivatives, which was resolved by column chromatography on silica gel. Elution with hexane–benzene (1:2.5) afforded the less polar isomer (**2a**), mp 115–116.5 °C, and further elution with hexane–benzene (1:3) gave the more polar isomer (**3a**). The configurations at C-28 of these isomers were determined to be 28*S* for the less polar compound and 28*R* for the more polar one by the modified method of Horeau using GLC.[5] These results suggested the configuration at the C-24 position to be 24*R* for the less polar and 24*S* for the more polar isomer in view of the established mechanism of hydroboration. The assignments were confirmed by the transformation of the two alcohols to the corresponding 24-ethylcholesterols. The 28-mesylates of **2a** or **3a** were reduced with $NaBH_4$–HMPA[6] to give the 3,5-cyclo derivatives of 24-ethylcholesterol (70%) accompanied by the $\Delta^{24(28)}$ analogues (30%), which were removed by epoxidation with *m*-chloroperbenzoic acid followed by silica gel chromatography. By treatment of the 3,5-cyclo derivative with acid, **2a** gave (24*R*)-24-ethylcholesterol (sitosterol) (**4a**), mp 136.5–138 °C (20% from **1a**), and **3a** gave (24*S*)-ethylcholesterol (clionasterol) (**5a**), mp 141–142.5 °C.

Hydroboration–oxidation of the 3,5-cyclo derivative of 24-methylenecholesterol (**1b**) afforded the less polar 28-ol (**2b**) and the more polar 28-ol (**3b**), which were separated from each other by preparative TLC, developing with benzene–ethyl acetate (25:1) four times. Reduction of both 28-mesylates with $LiAlH_4$ followed by acid treatment gave (24*R*)-24-methylcholesterol (campesterol) (**4b**), mp 160.5–161 °C (20% from **1b**), from the less polar 28-ol and (24*S*)-24-methylcholesterol (dihydrobrassicasterol) (**5b**), mp 158.5–160 °C (20% from **1b**), from the more polar one.

^{1}H NMR spectra (100 MHz) of the synthetic 24-ethyl and -methyl compounds are in good agreement with the published data.[7] ^{13}C NMR spectra of the C-24 isomers[8] were also found to be distinguishable from each other, and the spectra of **4a** and **4b** were identical with those of authentic sitosterol and campesterol, respectively. The signals of C-20, -21, -23, and -24 of the 24*R* isomers appear at higher field than those of the 24*S* isomers. Thus, ^{13}C NMR is also useful for the identification of C-24 alkylsterol isomers as ^{1}H NMR reported recently.[7]

Experimental Section

Melting points were determined on a hot stage microscope and are uncorrected. ^{1}H NMR spectra were recorded on a Hitachi R-24A (60 MHz) or a JOEL JNM-4H-100 (100 MHz) in $CDCl_3$ solution with Me_4Si as an internal standard. ^{13}C NMR spectra were determined with a JEOL PS/PFT-100 spectrometer at 25.2 MHz in $CDCl_3$. Mass spectra were recorded on a Shimadzu LKB-9000.

6β-Methoxy-3,5-cyclostigmast-24(28)-ene (1a). A mixture of 1.41 g of fucosterol, 40 mL of pyridine, and 1.4 g of *p*-toluenesulfonyl chloride was stirred at room temperature overnight. The reaction mixture was poured into cold water, and the resulting precipitate was collected by filtration, washed with water, and dried in vacuo. The tosylate (1.45 g) was used for the next step without purification. A mixture of 1.45 g of the tosylate, 200 mL of methanol, and 2.0 g of KOAc was refluxed for 3 h.[9] After most of the solvent was evaporated, the mixture was extracted with ethyl acetate. The organic layer was washed with saturated NaCl, dried over $MgSO_4$, and evapoarated. Chromatography (eluted with hexane) of the product on silica gel gave 1.05 g of **1a** as an oil: NMR (60 MHz) δ 0.2–0.7 (1 H, m, 3-H), 1.6 (3 H, d, J = 6.5 Hz, 29-Me), 2.75 (1 H, m, 6-H), 3.3 (3 H, s, MeO), 5.1 (1 H, q, J = 6.5 Hz, 28-H). Anal. Calcd for $C_{30}H_{50}O$: M^+ *m/e* 426.3861. Found: M^+ *m/e* 426.3871.

(24*R*,28*S*)- and (24*S*,28*R*)-6β-Methoxy-3,5-cyclostigmast-28-ol (2a and 3a). To a solution of 1.05 g of **1a** in 20 mL of dry THF was added 5 mL of BH_3–THF complex in benzene (~1 N) under a

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nitrogen atmosphere. After being stirred for 1 h, the solution was cooled in an ice bath and 15 mL of 10% NaOH and then 10 mL of 30% hydrogen peroxide were added. The solution was stirred at room temperature for 2 h and extracted with ether. The ether extract was washed with water and saturated NaCl and dried over $MgSO_4$. The solvent was evaporated in vacuo, and the residue was chromatographed on a silica gel column. Elution with hexane–benzene (1:2.5) gave 350 mg of the less polar alcohol **2a:** mp 115–116.5 °C; NMR (60 MHz) δ 1.15 (3 H, d, J = 6.5 Hz, 29-Me), 2.73 (1 H, m, 6-H), 3.27 (3 H, s, MeO), 3.7 (1 H, m, 28-H). Anal. Calcd for $C_{30}H_{52}O_2$: C, 81.02; H, 11.79. Found: C, 81.19; H, 11.76. Further elution with hexane–benzene (1:3) gave 300 mg of the more polar isomer **3a** as an oil: NMR (60 MHz) spectrum of **3a** was quite similar to that of **2a;** MS m/e 444 (M^+), 429 ($M^+ - Me$), 412 ($M^+ - MeOH$), 389 ($M^+ - C_4H_7$). Compound **2a** showed the same mass spectrum. Anal. Calcd for $C_{30}H_{52}O_2$: M^+ m/e 444.3966. Found: M^+ m/e 444.3963.

Application of Modified Horeau's Method to 2a and 3a. A solution of 5 mg of **2a** in 65 μL of pyridine was treated with 5 μL of α-phenylbutyric anhydride at room temperature overnight. To the solution was added 6 μL of (+)-α-methylbenzylamine, and the resulting precipitate was diluted with ethyl acetate and analyzed by GLC (OV-17 at 190 °C). **3a** (5 mg) was treated similarly. The ratio of the peak height on GLC with the shorted retention time to that with the longer one was 1.11:0.89 for the sample from **2a** and 0.92:1.08 for the sample from **3a.**

(24*R*)-24-Ethylcholesterol (Sitosterol) (4a). To a solution of 100 mg of **2a** in 3 mL of pyridine was added 80 μL of methanesulfonyl chloride (MsCl) with cooling in an ice bath. After being stirred for 1 h at room temperature, the reaction mixture was poured into ice water and extracted with ether. The organic layer was washed with dilute HCl, saturated $NaHCO_3$, and saturated NaCl and dried over $MgSO_4$. Evaporation of the solvent gave the mesylate as an oil: NMR (60 MHz) δ 1.39 (3 H, d, J = 6.5 Hz, 29-Me), 2.75 (1 H, m, 6-H), 2.95 (3 H, s, MeS), 3.29 (3 H, s, MeO), 4.9 (1 H, m, 28-H). The mesylate was used for the next step without purification.

A mixture of the mesylate, 2 mL of HMPA, and 50 mg of $NaBH_4$ was heated at 80 °C for 2 h under argon. After being cooled, the reaction mixture was added to cold water and extracted with ether. The extract gave an oily product which was found to contain 70% of the 3,5-cyclo derivative of **4a** and 30% of the elimination product (3,5-cyclo derivative of isofucosterol) by GLC analysis. To a solution of the crude product in 10 mL of CH_2Cl_2 was added 100 mg of m-chloroperbenzoic acid with cooling in an ice bath. After being stirred for 30 min at 0–5 °C, dilute NaOH was added to the reaction mixture and the mixture was extracted with CH_2Cl_2. The organic layer was washed with water and dried over $MgSO_4$, and the solvent was evaporated. The product was purified by silica gel chromatography to give 75 mg of the 3,5-cyclo derivative of **4a** (eluted with hexane–benzene, 2:1): NMR (60 MHz) δ 2.75 (1 H, m, 6-H), 3.30 (3 H, s, MeO).

A mixture of the product, 2 mL of dioxane, 0.7 mL of water, and a catalytic amount of p-TsOH was stirred at reflux for 3 h. Extraction with ether, the usual workup, and crystallization from methanol gave 60 mg of (24*R*)-24-ethylcholesterol (**4a**), mp 136.5–138 °C (lit.[7a] mp 139–140 °C).

(24*S*)-Ethylcholesterol (Clionasterol) (5a). When 100 mg of **3a** was treated in the same manner as described for **2a,** 55 mg of **5a** was obtained, mp 141–142.5 °C (from methanol) (lit.[7a] mp 142–143 °C).

6β-Methoxy-3,5-cycloergost-24(28)-ene (1b). When 495 mg of 24-methylenecholesterol was treated in the same manner as described for **1a,** 330 mg of **1b** was obtained as an oil: NMR (60 MHz) δ 0.2–0.7 (1 H, m, 3-H), 2.75 (1 H, m, 6-H), 3.3 (3 H, s, MeO), 4.7 (2 H, broad s, 28-H). Anal. Calcd for $C_{29}H_{48}O$: M^+ m/e 412.3705. Found: M^+ m/e 412.3717.

(24*R*)- and (24*S*)-6β-Methoxy-3,5-cycloergost-28-ol (2b and 3b). Hydroboration–oxidation of 50 mg of **1b** using the same procedure described for **1a** gave an epimeric mixture of **2b** and **3b,** which was separated by preparative TLC (four times of development) with benzene–ethyl acetate (25:1) to give 15 mg of the less polar alcohol **2b** and 12 mg of the more polar isomer **3b.** NMR (60 MHz) and mass spectra of the separated **2b** and **3b** are almost the same: NMR (60 MHz) δ 2.75 (1 H, m, 6-H), 3.29 (3 H, s, MeO), 3.53 (2 H, m, 28-H); MS m/e 430 (M^+), 415 ($M^+ - CH_3$), 398 ($M^+ - MeOH$), 375 ($M^+ - C_4H_7$).

(24*R*)-Methylcholesterol (Campesterol) (4b). Compound **2b** (15 mg) was converted to the corresponding mesylate by treatment with MsCl (10 μL)–pyridine (0.5 mL). To a suspension of 15 mg of $LiAlH_4$ in dry ether was added the mesylate in 1 mL of dry ether under argon. The mixture was refluxed for 3 h and cooled. Moist ether and dilute HCl were added to the mixture. Extraction with ether and the usual workup gave 13 mg of the product. Acid treatment of the product as described above gave 10 mg of **4b,** mp 160.5–161 °C (methanol) (lit. mp 159–160[7b] and 160–161 °C[7a]).

(24*S*)-Methylcholesterol (Dihydrobrassicasterol) (5b). When 12 mg of **3b** was treated in the manner described for **2b,** 8 mg of **5b** was obtained, mp 158.5–160 °C (from methanol) (lit. mp 157–158[7b] and 158–159 °C[7a]).

Registry No.—**1a,** 68844-30-4; **1b,** 68844-31-5; **2a,** 68844-32-6; **2a** mesylate, 68844-33-7; **2b,** 68844-34-8; **2b** mesylate, 68844-35-9; **3a,** 68889-64-5; **3b,** 6889-65-6; **4a,** 83-46-5; **4a** 3,5-cyclo derivative, 53139-46-1; **4b,** 474-62-4; **5a,** 83-47-6; **5b,** 4651-51-8; fucosterol, 17605-67-3; fucosterol tosylate, 68844-36-0; isofucosterol 3,5-cyclo derivative, 66461-40-3; 24-methylenecholesterol, 474-63-5.

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Bromination of 3-Cyclopentyl-2-butenolide

Fuk-Wah Sum and Larry Weiler*

Department of Chemistry, University of British Columbia Vancouver, British Columbia, Canada V6T 1W5

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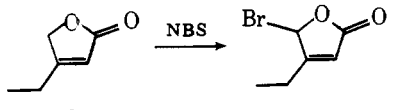
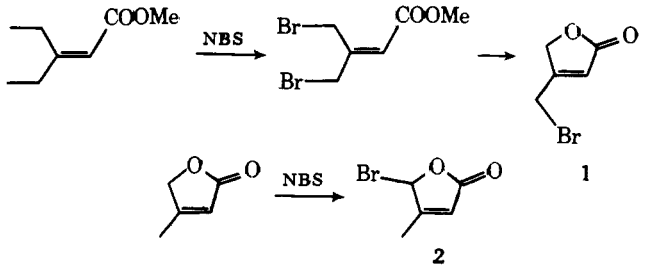
Recently Martin et al. reported the synthesis of the simple brominated butenolides **1** and **2** shown in Scheme I.[1] In particular, we note that the NBS bromination of the 3-methyl-2-butenolide occurs α to the oxygen. Steyn and co-workers had earlier found the same results, and in addition they found that 3-ethyl-2-butenolide (**3**) also underwent an NBS bromination α to the oxygen (eq 1).[2] We have investigated the bromination



(1)[2]

3

Scheme I. Synthesis of 3-Methyl Brominated Butenolides[1]



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