[Chem. Pharm. Bull. 30(4)1198—1203(1982)]

Syntheses of Steroids having a Bakkenolide-Type Spirolactone Ring. I. Synthesis of 4′-Methylenedihydrospiro-[5α-cholestane-3,3′(2′*H*)-furan]-2′-one

Kazuaki Kano, Koji Hayashi, and Hiroshi Mitsuhashi*

Faculty of Pharmaceutical Sciences, Hokkaido University, Kita 12-jo, Nishi 6-chome, Kita-ku, Sapporo, 060, Japan

(Received September 2, 1981)

Steroids having an α-spiro β-methylene γ-lactone structure, (3*R*) and (3*S*)-4′-methylenedihydrospiro-[5α-cholestane-3,3′(2′*H*)-furan]-2′-one, were synthesized from 5α-cholestan-3-one by means of the Knoevenagel reaction, conjugated addition of a cyanide ion, reduction of ester groups, hydrolysis and dehydration.

Keywords——bakkenolide; cholestane nucleus; hydrocyanation; Knoevenagel reaction; 4′-methylenedihydrospiro-[5α-cholestane-3,3′(2′*H*)-furan]-2′-one; β-methylene γ-lactone

Bakkenolides are sesquiterpene lactones isolated from *Petasites japonicus* (Sieb. *et* Zucc.) Maxim.[1] and several other species of the tribe Senecioneae (Compositae).[2] Bakkenolide A (I) has been reported to have cytotoxic activity.[3] Our unpublished data also indicated cytotoxicity of bakkenolide A against Ehrlich carcinoma. Many sesquiterpene lactones have been found to have cytotoxic activity. Most of them have a conjugated α-methylene group which has been suggested to be related to the cytotoxicity.[4] In spite of its similar cytotoxicity, bakkenolide A (I) does not contain such a conjugated system, but has an unconjugated β-methylene γ-lactone system. Thus, the cytotoxicity of compounds having such α-spiro β-methylene γ-lactone structures is of interest. Here we report the introduction of this structure into the cholestane system.

Several synthetic pathways have been reported for compounds having α-spiro β-methylene γ-lactone structure, including bakkenolide A (I).[5] In this report, we used a hydrocyanation reaction at a conjugated unsaturated ester for construction of the quaternary carbon center.

Knoevenagel reaction of cholestanone (II) and diethyl malonate using titanium tetrachloride and pyridine in tetrahydrofuran gave[6] the conjugated diester (III) in 59% yield after chromatographic separation and recrystallization. Hydrocyanation of the conjugated diester (III) was accomplished with potassium cyanide and ammonium chloride in aqueous dimethylformamide. After column chromatography of the reaction mixture, two diastereoisomeric cyanodiesters (IVa) and (IVb) were isolated in 45 and 25% yields, respectively. The yield of the decarbethoxylated product was increased when the reaction time was lengthened.

The cyanodiester (IVa) was then reduced with sodium borohydride and lithium chloride in diethylene glycol dimethyl ether, giving a mixture of the cyanodiol (Va) and the decarbethoxylated and reduced product (VIa). The mixture was hydrolyzed and cyclized directly by sulfuric acid treatment. By column chromatography, the lactone (VIIa) and the hydroxymethyllactone (VIIIa) were obtained in 21 and 48% yields, respectively. Similarly, VIIb and VIIIb were obtained from IVb in 5 and 26% yields, respectively. Nuclear magnetic resonance (NMR) spectra indicated that VIIIa and VIIIb are mixtures of diastereoisomers at the 4′ position, but further separation was not attempted.

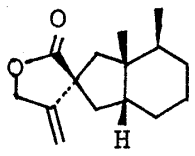
Then, dehydration of the hydroxymethyllactone (VIIIa) to the methylenelactone (IXa) was investigated. Treatment of VIIIa with phosphoryl chloride or thionyl chloride in pyridine gave the chloride (Xa). The dehydrated product (IXa) was not detected at this stage. The methylenelactone (IXa) was obtained by dehydrochlorination of the chloride (Xa) with potas-

sium-*tert*-butoxide in dimethylformamide in 40% overall yield from VIIIa. Alternatively, the hydroxymethyllactone (VIIIa) was treated with *o*-nitrophenyl selenocyanate and tri-*n*-butylphosphine.[7] The selenide (XIa) thus obtained was oxidized with hydrogen peroxide.[8] Decomposition of the resulting selenoxide the methylenelactone (IXa) in 88% overall yield after chromatographic purification. Similarly, the methylenelactone (IXb) was obtained from the hydroxymethyllactone (VIIIb) *via* the selenide (XIb) in 81% overall yield.

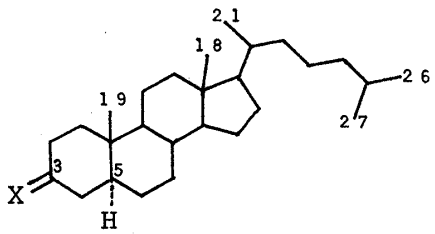
In the infrared (IR) spectra, IXa and IXb showed absorptions of unconjugated terminal methylene (IXa: 1660 cm^{-1}, IXb: 1665 cm^{-1}, I: 1665 cm^{-1}), and γ-lactone (IXa: 1755 cm^{-1},

Table I. ^1H NMR Chemical Shifts of 19-Methyl Protons

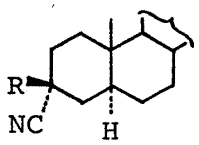
	VII	VIII	IX	X	XI
a	0.79	0.78	0.85	0.79	0.84
b	0.87	0.88	0.90	0.89	0.92



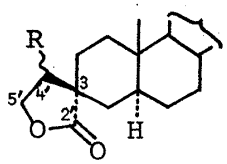
I

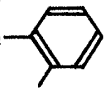


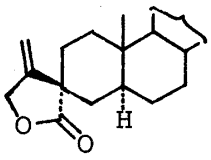
II : X=O
III: X=C($COOC_2H_5$)$_2$



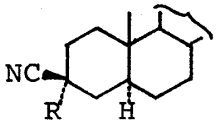
IVa: R=CH($COOC_2H_5$)$_2$
Va : R=CH(CH_2OH)$_2$
VIa: R=CH_2CH_2OH



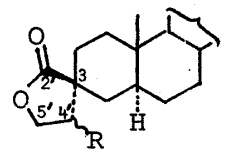
VIIa : R=H
VIIIa: R=CH_2OH
Xa : R=CH_2Cl
XIa : R=CH_2Se- 

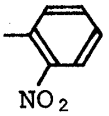


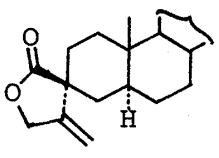
IXa



IVb: R=CH($COOC_2H_5$)$_2$
Vb : R=CH(CH_2OH)$_2$
VIb: R=CH_2CH_2OH



VIIb : R=H
VIIIb: R=CH_2OH
Xb : R=CH_2Cl
XIb : R=CH_2Se- 



IXb

Fig. 1

IXb: 1770 and 1760 cm^{-1}, I: 1765 cm^{-1}). In the ^1H NMR spectra, IXa and IXb showed signals of two terminal methylene protons (IXa: δ 5.05 ppm (2H), IXb: δ 5.09 and 5.40 ppm (each 1H), I: δ 5.02 and 5.11 ppm (each 1H)), and allylic protons bonded to oxygen atoms (IXa: δ 4.78 ppm (2H), IXb: δ 4.71 ppm (2H), I: 4.76 ppm (2H)). Terminal methylene protons and allylic protons were coupled with a coupling constant of *ca.* 2 Hz.

Stereochemistries of the 3-positions of these compounds were determined from the chemical shifts of the 19-methyl protons in the ^1H NMR spectra. The effect of 2′-carbonyl groups on the chemical shifts of the 19-methyl protons can be seen in the 4′-nonsubstituted lactones (VIIa) and (VIIb). The signal of the 19-methyl protons of VIIb appeared further downfield than that of VIIa, indicating that deshielding by the 2′-carbonyl group was larger at the 19-methyl protons of VIIb than at those of VIIa. Similar results were obtained for VIII, IX, X and XI (Table I). Moreover, the effect of the 4′-methylene group of the chemical shifts of 19-methyl protons is indicated by the differences between those of the 4′-methylenlactones (IXa), (IXb) and those of the 4′-unsubstituted lactones (VIIa), (VIIb). This value was larger in IXa ($\Delta\delta$=0.06 ppm) than in IXb ($\Delta\delta$=0.03 ppm). These data indicate that in IXa, the methylene group exists on the same side of the molecule as the 19-methyl group, while the carbonyl group is on the opposite side, and in IXb, the methylene group is on the opposite side and the carbonyl group is on the same side. Accordingly, IXa has 3*R* configuration and IXb has 3*S*.

Assays of the cytotoxic activities of the β-methylene lactones (IXa) and (IXb) are in progress.

Experimental

Melting points were measured on a Ernst Leitz (Wetzlar) machine and are uncorrected. Specific rotations were measured with a JASCO DIP-4 digital polarimeter in chloroform solutions. IR spectra were recorded on a Hitachi 215 spectrometer in Nujol suspensions, ultraviolet (UV) spectra on a Shimadzu UV-220 spectrometer, ^1H NMR spectra on a JEOL FX-100 spectrometer at 100 MHz in deuteriochloroform solutions with tetramethylsilane as an internal standard, and mass spectra (MS) on a JMS D-300 mass spectrometer.

Column chromatographies were carried out on Merck silica gel 60 and preparative thin layer chromatographies (TLC) on 20 cm × 20 cm plates with 0.75 mm layers of Merck silica gel PF_{254}.

Diethyl 5α-Cholestan-3-ylidenemalonate (III)——A solution of 5 ml of titanium tetrachloride in 22 ml of carbon tetrachloride was added dropwise to 40 ml of absolute tetrahydrofuran with stirring under ice cooling and under a nitrogen atmosphere. To this yellow emulsion, 3.1 ml of diethyl malonate and 3.81 g of 5α-cholestan-3-one (III) were added, and then 3.25 ml of pyridine in 7 ml of absolute tetrahydrofuran was added dropwise. The resulting brick-red emulsion was stirred at room temperature under a nitrogen atmosphere for three days. Water was added to the reaction mixture, and the whole was stirred until all the solids has dissolved, then extracted with ether. The ethereal extract was washed with 2 N HCl aq., 5% $NaHCO_3$ aq., and sat. NaCl aq., dried over Na_2SO_4 and evaporated to dryness. The residue was chromatographed on silica gel. Elution with benzene gave 3.87 g of crude product. Recrystallization from ethanol gave 3.07 g (58.9% yield) of diethyl 5α-cholestan-3-ylidenemalonate (III) as colorless plates. mp 108.5—110°C. $[\alpha]_D^{18}$ +19° (c=0.20). IR ν_{max}^{Nujol} cm^{-1}: 1715, 1630, 1365, 1265, 1230, 1215, 1185, 1150, 1095, 1055, 1035. UV λ_{max}^{EtOH} nm: 227 (ε 13000). NMR δ: 0.65 (3H, s, CH_3-18), 0.86 (6H, d, J=6 Hz, CH_3-26 and 27), 0.90 (3H, s, CH_3-19), 1.28 (6H, t, J=7 Hz, C<u>H</u>$_3$CH$_2$O–), 4.22 (4H, q, J=7 Hz, CH$_3$C<u>H</u>$_2$O–). MS *m/z*: 528 (M^+), 482 (base peak), 467, 454, 436. *Anal.* Calcd for $C_{34}H_{56}O_4$: C, 77.22; H, 10.67. Found: C, 77.22; H, 10.86.

Diethyl (3-Cyano-5α-cholestan-3-yl)malonate (IV)——A mixture of 2.01 g of diethyl 5α-cholestan-3-ylidenemalonate (III), 305 mg of ammonium chloride, 508 mg of potassium cyanide, 40 ml of dimethylformamide and 5 ml of water was heated on an oil bath at 100°C for 1.5 h. After cooling, the reaction mixture was poured into water and extracted with ether. The ether extract was washed with water, 2 N HCl aq. 5% $NaHCO_3$ aq. and sat. NaCl aq., dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel. Elution with benzene gave 582 mg of diethyl (3α-cyano-5α-cholestan-3β-yl)malonate (IVa). Rechromatography of the mixed fraction gave a second crop of 524 mg of IVa. Recrystallization of 857 mg of crude IVa from *n*-hexane gave 744 mg (45.4% yield) of colorless needles, mp 111.5—113°C/138.5—140°C. $[\alpha]_D^{20}$ +20° (c=0.23). IR ν_{max}^{Nujol} cm^{-1}: 2240, 1755, 1730, 1305, 1255, 1235, 1215, 1210, 1175, 1095, 1030. NMR δ: 0.64 (3H, s, CH_3-18), 0.76 (3H, s, CH_3-19), 0.87 (6H, d, J=6 Hz, CH_3-26, 27), 1.29 (6H, t, J=7 Hz, C<u>H</u>$_3$-CH$_2$O–), 3.40 (1H, s, –C<u>H</u>(COOEt)$_2$), 4.25 (4H, q, J=7 Hz, CH$_3$C<u>H</u>$_2$O–). MS *m/z*: 555 (M^+), 540, 400,

160 (base peak). *Anal.* Calcd for $C_{35}H_{57}O_4N$: C, 75.63; H, 10.34; N, 2.52. Found: C, 75.52; H, 10.53; N, 2.63.

Further elution gave 534 mg (25.3% yield) of diethyl (3β-cyano-5α-cholestan-3α-yl)malonate (IVb) as colorless needles, mp 101.5—103°C from methanol. $[\alpha]_D^{22}$ +27° (c=0.20). IR ν_{max}^{Nujol} cm^{-1}: 2250, 1750, 1725, 1305, 1280, 1270, 1250, 1200, 1150, 1100, 1035, 740. NMR δ: 0.64 (3H, s, CH_3-19), 0.85 (3H, s, CH_3-18), 0.87 (6H, d, J=6 Hz, CH_3-26, 27), 1.30 (6H, t, J=7 Hz, $C\underline{H}_3CH_2O$–), 3.91 (1H, s, –C$\underline{H}$(COOEt)$_2$), 4.27 (4H, q, J=7 Hz, $CH_3C\underline{H}_2O$–). MS m/z: 555 (M^+), 540, 400, 160 (base peak). *Anal.* Calcd for $C_{35}H_{57}O_4N$: C, 75.63; H, 10.34; N, 2.52. Found: C, 75.82; H, 10.41; N, 2.54.

3β-(2-Hydroxy-1-hydroxymethylethyl)-5α-cholestane-3α-carbonitrile (Va)——A mixture of 14.3 mg of sodium borohydride, 16.8 mg of lithium chloride and 2 ml of diglyme was stirred for 30 minutes. Next, 102 mg of diethyl (3α-cyano-5α-cholestan-3β-yl)malonate (IVa) was added and the whole was heated at 100°C on an oil bath for 3 h. After cooling, the reaction mixture was diluted with ether, washed with 2 N HCl aq., 5% $NaHCO_3$ aq. and sat. NaCl aq., dried over Na_2SO_4 and concentrated. Separation of the residue by preparative TLC gave 28.1 mg (34.7% yield) of 3β-(2-hydroxyethyl)-5α-cholestane-3α-carbonitrile (VIa), $[\alpha]_D^{18}$ +23° (c=1.0). IR ν_{max}^{Nujol} cm^{-1}: 3450—3280, 2225, 1045, 720. NMR δ: 0.64 (3H, s, CH_3-18), 0.76 (3H, s, CH_3-19), 0.87 (6H, d, J=6 Hz, CH_3-26, 27), 0.90 (3H, d, J=6 Hz, CH_3-21), 1.83 (2H, t, J=7 Hz, $HOCH_2$-$C\underline{H}_2$–), 3.89 (2H, t, J=7 Hz, $HOC\underline{H}_2CH_2$–). MS m/z: 441 (M^+), 426, 301, 286 (base peak), 272, and 49.9 mg (57.6% yield) of 3β-(2-hydroxy-1-hydroxymethylethyl)-5α-cholestane-3α-carbonitrile (Va), $[\alpha]_D^{16}$ +24° (c=1.1). IR ν_{max}^{Nujol} cm^{-1}: 3300, 2225, 1040. NMR δ: 0.64 (3H, s, CH_3-18), 0.76 (3H, s, CH_3-19), 0.86 (6H, d, J=6 Hz, CH_3-26, 27), 0.89 (3H, d, J=6 Hz, CH_3-21), 4.03 (4H, m, $(HOC\underline{H}_2)_2CH$–). MS m/z: 471 (M^+), 456, 440, 415 (base peak), 331, 316, 302, 128.

(3*R*)-4′-hydroxymethyldihydrospiro-[5α-cholestane-3,3′(2′*H*)-furan]-2′-one (VIIIa)——Reduction of 600 mg of diethyl (3α-cyano-5α-cholestan-3β-yl)malonate (IVa) was carried out as described above. The mixture thus obtained was dissolved in 20 ml of diglyme and 20 ml of water, then 20 ml of sulfuric acid was added and the whole was refluxed for 3 h. After cooling, this mixture was poured into water, extracted with ether, washed with 5% $NaHCO_3$ aq. and sat. NaCl aq., dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel. Elution with chloroform gave a fraction containing VIIa, which was rechromatographed. Elution with benzene–hexane (1: 1) gave 100 mg (20.9% yield) of (3*R*)-dihydrospiro-[5α-cholestane-3,3′(2′*H*)-furan]-2′-one (VIIa), colorless needles from chloroform-methanol, mp 181.5—183°C. $[\alpha]_D^{20}$ +30° (c=0.21). IR ν_{max}^{Nujol} cm^{-1}: 1755, 1215, 1135, 1115, 1025, 960. NMR δ: 0.64 (3H, s, CH_3-18), 0.79 (3H, s, CH_3-19), 0.87 (6H, d, J=6 Hz, CH_3-26, 27), 2.04 (2H, t, J=7 Hz, H-4′), 4.23 (2H, t, J=7 Hz, H-5′). MS m/z: 442 (M^+), 427, 329, 302, 287 (base peak), 273, 262. *Anal.* Calcd for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 81.36; H, 11.58.

Further elution with 5% acetone in chloroform gave 248 mg (48.6% yield) of (3*R*)-4′-hydroxymethyldihydrospiro-[5α-cholestane-3,3′(2′*H*)-furan]-2′-one (VIIIa), colorless powder from chloroform–methanol, mp 227—231°C. $[\alpha]_D^{20}$ +38° (c=0.21). IR ν_{max}^{Nujol} cm^{-1}: 3350, 1755, 1220, 1200, 1155, 1130, 1115, 1035, 1020, 720. NMR δ: 0.64 (3H, s, CH_3-18), 0.78 (3H, s, CH_3-19), 0.86 (6H, d, J=7 Hz, CH_3-26, 27), 2.29 (1H, m, H-4′), 3.72 (1H, dd, J=8, 11 Hz) and 3.83 (1H, dd, J=5, 11 Hz, –C$\underline{H}_2$OH), 4.1 (1H, m) and 4.38 (1H, dd, J=7, 9 Hz, H-5′). MS m/z: 472 (M^+, base peak), 457, 359, 332, 329, 317, 303. *Anal.* Calcd for $C_{31}H_{52}O_3$: C, 78.76; H, 11.09. Found: C, 78.83; H, 11.11.

(3*R*)-4′-Chloromethyldihydrospiro-[5α-cholestane-3,3′(2′*H*)-furan]-2′-one (Xa)——A) Phosphoryl chloride (0.2 ml) was added to a solution of 45.5 mg of (3*R*)-4′-hydroxymethyldihydrospiro-[5α-cholestane-3,3′(2*H*)-furan]-2′-one (VIIIa) in 2 ml of pyridine under ice cooling and the mixture was stirred for 2.5 h. Then this mixture was poured into water and extracted with ether. The ether extract was washed with 2 N HCl aq., 5% $NaHCO_3$ aq. and sat. NaCl aq., dried over Na_2SO_4 and concentrated. The residue was recrystallized from chloroform–methanol to give 35.0 mg (74.0% yield) of (3*R*)-4′-chloromethyldihydrospiro-[5α-cholestane-3,3′(2′*H*)-furan]-2′-one (Xa) as a colorless powder, mp 209—211°C. $[\alpha]_D^{21}$ +40° (c=0.22). IR ν_{max}^{Nujol} cm^{-1}: 1775, 1030. NMR δ: 0.64 (3H, s, CH_3-18), 0.79 (3H, s, CH_3-19), 0.87 (6H, d, J=6 Hz, CH_3-26, 27), 0.90 (3H, d, J=6 Hz, CH_3-21), 2.48 (1H, m, H-4′), 3.50 (1H, dd, J=11, 10 Hz) and 3.68 (1H, dd, J=11, 5 Hz, –CH_2Cl), 4.1 (1H, m) and 4.42 (1H, dd, J=9, 7 Hz, H-5′). MS m/z: 490 (M^+), 475, 350, 335 (base peak), 321. *Anal.* Calcd for $C_{31}H_{52}ClO_2$: C, 75.80; H, 10.46; Cl, 7.22. Found: C, 75.49; H, 10.42; Cl, 7.25.

B) Thionyl chloride (0.12 ml) was added to a solution of 25.4 mg of VIIIa in 1 ml of pyridine under ice cooling and the mixture was stirred for 1 h. The product was identified as Xa by TLC comparison with an authentic sample.

(3*R*)-4′-2-Nitrophenylselenomethyl)dihydrospiro-[5α-cholestane-3,3′(2′*H*)-furan]-2′-one (XIa)——To a solution of 49.1 mg of (3*R*)-4′-hydroxymethyldihydrospiro-[5α-cholestane-3,3′(2′*H*)-furan]-2′-one (VIIIa) and 34.7 mg of *o*-nitrophenyl selenocyanate in absolute tetrahydrofuran, a solution of 0.4 ml of tri-*n*-butylphosphine in 1 ml of absolute tetrahydrofuran was added dropwise under a nitrogen atmosphere. This mixture was stirred at 50°C for 30 minutes, then the solvent was evaporated off under reduced pressure, and the residue was chromatographed on silica gel. Elution with benzene gave 52.3 mg of (3*R*)-4′-(2-nitrophenylselenomethyl)dihydrospiro-[5α-cholestane-3,3′(2′*H*)-furan]-2′-one (XIa). Recrystallization from chloroform–methanol gave 46.5 mg (68.2% yield) of a yellow amorphous material. On heating, it became crystalline at 195—196.5°C and melted at 205—207.5°C. $[\alpha]_D^{24}$ +29° (c=0.21). UV $\lambda_{max}^{Dioxane}$ nm: 254 (ε 11000), 384

(ε 3190). IR ν_{max}^{Nujol} cm^{-1}: 1740, 1585, 1560, 1510, 1310, 1300, 1140, 1095, 1035, 1020, 850, 780, 720. NMR δ: 0.65 (3H, s, CH_3-18), 0.84 (3H, s, CH_3-19), 0.87 (6H, d, J=6 Hz, CH_3-26, 27), 0.91 (3H, d, J=6 Hz, CH_3-21), 2.5 (1H, m, H-4'), 2.79 (1H, t, J=11 Hz) and 3.16 (1H, dd, J=11, 3 Hz, $-CH_2Se$), 4.0 (1H, m) and 4.46 (1H, dd, J=9, 7 Hz, H-5'), 7.48 (3H, m) and 8.31 (1H, dd, J=8, 1 Hz, aromatic protons). MS m/z: 657 (M^+), 627, 454, 439, 398, 341, 329, 315, 299, 287, 285, 43 (base peak). *Anal.* Calcd for $C_{37}H_{55}NO_4Se$: C, 67.66; H, 8.44. Found: C, 67.60; H, 8.44.

(3*R*)-4'-Methylenedihydrospiro-[5α-cholestane-3,3'(2'*H*)-furan]-2'-one (IXa)——A) (from the chloride Xa). Potassium *tert*-butoxide was prepared from 71.3 mg of potassium and 2 ml of *tert*-butanol. After the excess *tert*-butanol had been distilled off, 2 ml of dimethyl sulfoxide and 25.0 mg of (3*R*)-4'-chloromethyldihydrospiro-[5α-cholestane-3,3'(2'*H*)-furan]-2'-one (Xa) were added and the whole was heated on an oil bath at 100°C under a nitrogen atmosphere overnight. The reaction mixture was acidified with 2 N HCl. aq. and extracted with ether. The ether extract was washed with 2 N HCl aq. and sat. NaCl aq. The solvent was evaporated off, and the residue was dissolved in 2 ml of diglyme, then 1 ml of water and 1 ml of conc. sulfuric acid were added and the mixture was stirred at 100°C for 30 minutes. Water was added to the reaction mixture and it was extracted with ether. The ether extract was washed with water, 5% $NaHCO_3$ aq. and sat. NaCl aq., dried over Na_2SO_4 and concentrated. The residue was purified by preparative TLC. Development with benzene–hexane (1: 1) and extraction gave 12.6 mg (54.4% yield) of (3*R*)-4'-methylenedihydrospiro-[5α-cholestane-3,3'(2'*H*)-furan]-2'-one (IXa).

B) (from the selenide XIa). The selenide XIa [prepared from 101 mg of (3*R*)-4'-hydroxymethyldihydrospiro-[5α-cholestane-3,3'(2'*H*)-furan]-2'one (VIIIa) as described above] was dissolved in 3 ml of tetrahydrofuran and the solution was cooled on an ice bath. To this solution, 5 ml of 30% hydrogen peroxide was added and the whole was stirred at 30°C overnight. Water was added to the reaction mixture and it was extracted with ether. The ether extract was washed with 5% $NaHCO_3$ aq. and sat. NaCl aq., dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel. Elution with benzene gave 89.6 mg of a yellow material. Recrystallization from chloroform–methanol gave 85.7 mg (88.2% yield) of (3*R*)-4'-methylenedihydrospiro-[5α-cholestane-3,3'(2'*H*)-furan]-2'-one (IXa) as colorless plates, mp 162.5—164°C, $[\alpha]_D^{22}$ +44° (c=0.20). IR ν_{max}^{Nujol} cm^{-1}: 1755, 1660, 1350, 1130, 1030, 905. NMR δ: 0.65 (3H, s, CH_3-18), 0.85 (3H, s, CH_3-19), 0.87 (6H, d, J=6 Hz, CH_3-26, 27), 0.91 (3H, d, J=6 Hz, CH_3-21), 4.78 (2H, t, J=2 Hz, H-5'), 5.05 (2H, t, J=2 Hz, CH_2=). MS m/z: 454 (M^+), 439, 398, 341, 329, 314, 299 (base peak), 287, 285. *Anal.* Calcd for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found: C, 81.93; H, 11.19.

(3*S*)-4'-Hydroxymethyldihydrospiro-[5α-cholestane-3,3'(2*H*)-furan]-2'-one (VIIIb)——Reduction of 1.52 g of diethyl (3β-cyano-5α-cholestane-3α-yl)malonate (IVb) was carried out as described for the synthesis of Va using 1.04 g of sodium borohydride, 1.73 g of lithium chloride and 20 ml of diglyme. The mixture was cooled, then 30 ml each of 10% sulfuric acid and conc. sulfuric acid were added carefully and the whole was heated at 100°C for 3 h, then cooled. Water was added to the reaction mixture and solids that separated were collected by filtration. This product was dissolved in chloroform and column-chromatographed on silica gel. Elution with benzene gave 79.6 mg of (3*S*)-4'-chloromethyldihydrospiro-[5α-cholestane-3,3'(2'*H*)-furan]-2'-one (Xb). Recrystallization from methanol gave 70.8 mg (5.27% yield) of a colorless powder, mp 143—147°C. $[\alpha]_D^{22}$ +25° (c=0.25). IR ν_{max}^{Nujol} cm^{-1}: 1760, 1740, 1215, 1160, 1130, 1120, 1050, 995. NMR δ: 0.65 (3H, s, CH_3-18), 0.87 (6H, d, J=6 Hz, CH_3-26, 27), 0.89 (3H, s, CH_3-19), 2.72 (1H, m, H-4'), 3.42 (1H, m) and 3.67 (1H, m, $-CH_2Cl$), 4.38 (2H, m, H-5'). MS m/z: 490 (M^+), 475, 350, 335, 108 (base peak).

Further elution with benzene gave 73.7 mg of (3*S*)-dihydrospiro-[5α-cholestane-3,3'(2'*H*)-furan]-2'-one (VIIb). Recrystallization from methanol gave 63.3 mg (5.23% yield) of colorless plates, mp 203.5—205.5°C. $[\alpha]_D^{24}$+29° (c=0.21). IR ν_{max}^{Nujol} cm^{-1}: 1750, 1740, 1215, 1210, 1180, 1020, 730. NMR δ: 0.65 (3H, s, CH_3-18), 0.87 (6H, d, J=6 Hz, CH_3-26, 27), 0.87 (3H, s, CH_3-19), 2.16 (2H, t, J=7 Hz, H-4'), 4.25 (2H, t, J=7 Hz, H-5'). MS m/z: 442 (M^+, base peak), 427, 302, 287. *Anal.* Calcd for $C_{31}H_{52}O_3$: C, 81.39; H, 11.38. Found: C, 81.35; H, 11.48.

Further elution with 10% acetone in benzene gave 388 mg of (3*S*)-4'-hydroxymethyldihydrospiro-[5α-cholestane-3,3'(2'*H*)-furan]-2'-one (VIIIb). Recrystallization from methanol gave 333 mg (25.8% yield) of colorless powder, mp 207.5—211°C. $[\alpha]_D^{24}$ +34° (c=0.24), IR ν_{max}^{Nujol} cm^{-1}: 3420, 1725, 1225, 1180, 1125, 1010, 720. NMR δ: 0.65 (3H, s, CH_3-18), 0.87 (6H, d, J=6 Hz, CH_3-26, 27), 0.88 (3H, s, CH_3-19), 2.54 (1H, m, H-4'), 3.58 (1H, m) and 3.77 (1H, m, $-C\underline{H}_2OH$), 4.37 (2H, m, H-5'). MS m/z: 472 (M^+, base peak), 457, 332, 317. *Anal.* Calcd for $C_{31}H_{52}O_3$: C, 78.76; H, 11.09. Found: C, 78.68; H, 10.97.

(3*S*)-4'-(2-Nitrophenylselenomethyl)dihydrospiro-[5α-cholestane-3,3'(2'H)-furan]-2'one (XIb)——*o*-Nitrophenylselenylation of 50.5 mg of (3*S*)-4'-hydroxymethyldihydrospiro-[5α-cholestane-3,3'(2'*H*)-furan]-2'-one (VIIIb) was carried out as described the synthesis of the 3*R*-isomer (XIa) using 39.6 mg of *o*-nitrophenyl selenocyanate, 0.05 ml of tri-*n*-butylphosphine and 1 ml of absolute tetrahydrofuran to afford 72.4 mg of (3*S*)-4'-(2-nitrophenylselenomethyl)dihydrospiro-[5α-cholestane-3,3'(2'*H*)-furan]-2'-one (XIb). Recrystallization from methanol gave 60.5 mg (86.2% yield) of a yellow amorphous material. On heating, it became crystalline at *ca.* 110—120°C and melted at 177.5—179.5°C. $[\alpha]_D^{23}$ +15° (c=0.23). IR ν_{max}^{Nujol} cm^{-1}: 1760, 1740, 1585, 1560, 1510, 1315, 1300, 1170, 1035, 980, 850, 780, 725. UV $\lambda_{max}^{Dioxane}$ nm: 254 (ε 13100), 384 (ε 3820). NMR δ: 0.67 (3H, s, CH_3-18), 0.88 (3H, d, J=6 Hz, CH_3-26, 27), 0.92 (3H, s, CH_3-19), 2.68 (2H, m, H-4' and $-C\underline{H}HSe-$), 3.19 (1H, m, $-CH\underline{H}Se-$), 4.35 (2H, broad s, H-5'), 7.52 (3H, m) and 8.40 (1H, d, J=

$J=8$ Hz, aromatic protons). MS m/z: 657 (M^+), 627, 454, 439, 426, 341, 315, 299, 43 (base peak). *Anal.* Calcd for $C_{37}H_{55}NO_4Se$: C, 67.66; H, 8.44. Found: C, 67.46; H, 8.58.

(3*S*)-4′-Methylenedihydrospiro-[5α-cholestane-3,3′(2′*H*)-furan]-2′-one (IXb)——The proceduredescribed for the synthesis of the 3*R*-isomer, (IXa) was used, but without purification of the intermediate XIb. From 41.2 mg of *o*-nitrophenyl selenocyanate, 0.05 ml of tri-*n*-butylphosphine, 1 ml of absolute tetrahydrofuran, 1 ml of 30% hydrogen peroxide, and 51.5 mg of (3*S*)-4′-hydroxymethyldihydrospiro-[5α-cholestane-3,3′(2′*H*)-furan]-2′-one (VIIIb), we obtained 46.3 mg of (3*S*)-4′-methylenedihydrospiro-[5α-cholestane-3,3′(2′*H*)-furan]-2′-one (IXb) as a yellow mass. Recrystallization for methanol gave 40.3 mg (81.4% yield) of a colorless powder, mp 173—175.5°C. $[\alpha]_D^{23}$ +26° ($c=0.23$), IR ν_{max}^{Nujol} cm^{-1}: 1770, 1760, 1665, 1345, 1165, 1120, 1025, 905, 895. NMR δ: 0.66 (3H, s, CH_3-18), 0.87 (6H, d, $J=6$ Hz, CH_3-26, 27), 0.90 (3H, s, CH_3-19), 4.71 (2H, t, $J=2$ Hz, H-5′), 5.09 (1H, t, $J=2$ Hz) and 5.40 (1H, t, $J=2$ Hz, CH_2=), MS m/z: 454 (M^+), 439, 426, 314, 299, 43 (base peak). *Anal.* Calcd for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found: C, 82.06; H, 11.03.

Acknowledgement We are grateful to Miss A. Maeda for elemental analyses, to Mrs. K. Tsuta for NMR measurements, and to Miss S. Nishida for mass spectral measurements.

References and Notes

1) *a*) N. Abe, R. Onoda, K. Shirahata, T. Kato, M.C. Woods, and Y. Kitahara, *Tetrahedron Lett.*, **1968**, 369; N. Abe, R. Onoda, K. Shirahata, T. Kato, M.C. Woods, Y. Kitahara, K. Ro, and T. Kurihara, *Tetrahedron Lett.*, **1968**, 1993; K. Shirahata, N. Abe, T. Kato, and Y. Kitahara, *Bull. Chem. Soc. Jpn.*, **41**, 1732 (1968); Y. Kitahara, N. Abe, T. Kato, and K. Shirahata, *Nippon Kagaku Zasshi*, **90**, 221 (1969); K. Shirahata, T. Kato, Y. Kitahara, and N. Abe, *Tetrahedron*, **25**, 3179 (1969); *b*) K. Naya, I. Takagi, M. Hayashi, S. Nakamura, M. Kobayashi, and S. Katsumura, *Chem. Ind.* (London), **1968**, 318; K. Naya, M. Kawai, M. Naito, and T. Kasai, *Chem. Lett.*, **1972**, 241; K. Naya, M. Hayashi, I. Takagi, S. Nakamura, and M. Kobayashi, *Bull. Chem. Soc. Jpn.*, **45**, 3673 (1972).
2) N.H. Fischer, E.J. Olivier, and H.D. Fischer, *Fortschr. Chem. Org. Naturstoffe*, **38**, 257 (1979) and references cited therein.
3) F.R. Jamieson, E.H. Reid, B.P. Turner, and A.T. Jamieson, *Phytochemistry*, **15**, 1713 (1976); B. Hladon, B. Drozds, M. Holub, P. Szafarek, and O. Klimaszewska, *Pol. J. Pharmacol. Pharm.*, **30**, 611 (1978).
4) K.-H. Lee, E.-S. Huang, C. Piantadosi, J.S. Pagano, and T.A. Geissman, *Cancer Res.*, **31**, 1649 (1971); S.M. Kupchan, M.A. Eakin, and A.M. Thomas, *J. Med. Chem.*, **14**, 1147 (1971); I.H. Hall, K.-H. Lee, C.O. Starnes, S.A. Eigenbaly, T. Ibuka, Y.-S. Wu, T. Kimura, and M. Haruna, *J. Pharm. Sci.*, **67**, 1235 (1978).
5) K. Hayashi, H. Nakamura, and H. Mitsuhashi, *Chem. Pharm. Bull.*, **21**, 2806 (1973); D.A. Evans and C.L. Sims, *Tetrahedron Lett.*, **1973**, 4691; D.A. Evans, C.L. Sims, and G.C. Andrews, *J. Am. Chem. Soc.*, **99**, 5453 (1977); S.F. Campbell, M.G. Constantino, T.J. Brocksom, and N. Petraganani, *Synth. Commun.*, **5**, 353 (1975); N. Petraganani, T.J. Brocksom, H.M.C. Ferraz, and M.G. Constantino, *Synthesis*, **1977**, 1125, T.J. Brocksom, M.G. Constantino, and H.M.C. Ferraz, *Synth. Commun.*, **7**, 483 (1977); M. Bortolussi, R. Bloch, and J.M. Conia, *Bull. Soc. Chim. Fr.*, **1975**, 2727; E. Wenkert, M.E. Alonso, B.L. Buckwalter, and K.J. Chou, *J. Am. Chem. Soc.*, **99**, 4778 (1977).
6) W. Lehnert, *Tetrahedron*, **29**, 635 (1973).
7) P.A. Grieco, S. Gilman, and M. Nishizawa, *J. Org. Chem.*, **41**, 1485 (1976).
8) K.B. Sharpless and M.W. Young, *J. Org. Chem.*, **40**, 947 (1975).