Synthesis of 26-Halo-, 26-(Phenylseleno)-, and 26-Indolylcholesterol Analogues

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Diosgenin was converted to 26-hydroxycholesterol. Treatment of the 26-hydroxycholesterol with $(C_6H_5)_3P$ and *N*-bromo- or *N*-iodosuccinimide gave the 26-bromide and 26-iodide, respectively. Alkylation of 3-methylindole with 26-bromocholesterol 3-silyl ether gave the 26-(3-methylindol-1-yl)cholesterol. Reaction of cholesta-5,25-dien-3β-ol acetate with phenylselenium bromide and potassium acetate gave 26-(phenylseleno)cholest-5-ene-3β,25-diol diacetate and 26-(phenylseleno)cholest-5-ene-3β,25-diol 3-acetate.

For ongoing projects, we required cholesterol analogs substituted with bromine, iodine, selenium and an N-3-methyl indole moiety at C-26. The synthesis of these compounds is the subject of this paper.

Discussion

Clemmensen reduction[1] (Zn/Hg–HCl/EtOH) of diosgenin (1) furnished cholest-5-ene-3β,16β,26-triol (**2**, tetrahydrodiosgenin) in 50–60% yield (Chart I). The NMR spectrum of **2** showed a doublet at δ 3.43 (J = 6 Hz) for the 26-methylene (CH_2OH) protons, as expected. Selective oxidation[2] of the triol **2** (chromium trioxide in aqueous acetic acid in the presence of sodium acetate) gave a mixture of two products **3** and **4** which were resolved chromatographically. The infrared spectrum of the less polar compound (**3**) showed bands at 1720 cm^{-1} for the 26-aldehyde and at 1736 cm^{-1} for the 16-ketone. The NMR spectrum showed a doublet at δ 9.6 for an aldehyde proton as expected for the 25-formyl-3-hydroxycholest-5-en-16-one (**3**). The IR spectrum of the more polar major product (**4**, 65%) showed a band at 1736 cm^{-1} for the 16-ketone, and its NMR spectrum exhibited a doublet at δ 3.48 (J = 6 Hz) for the 26-methylene protons.

Wolff–Kishner reduction (Huang–Minlon modification) of the 16-ketone 3,26-diol **4** gave 26-hydroxycholesterol (**5**) in good yield. The obtained **5** was identical with an authentic sample[3] prepared from kryptogenin (**6**).

Primary hydroxyl groups can be selectively displaced with bromine or iodine by treatment with triphenylphosphine and *N*-bromosuccinimide or *N*-iodosuccinimide,[4] respectively. Accordingly, 26-hydroxycholesterol (**5**) was treated with a mixture of *N*-bromosuccinimide and triphenylphosphine in dimethylformamide to yield 26-bromocholesterol (**7a**) in 65–70% yield. The product gave a strong positive Beilstein test, and its NMR spectrum showed a doublet at δ 3.32 (J = 6 Hz) for the 26-methylene protons.

Similarly, treatment of **5** with *N*-iodosuccinimide and triphenylphosphine furnished 26-iodocholesterol [**7b**; 55–60% yield, NMR doublet at δ 3.2 (J = 6 Hz) for the 26-methylene protons)]. An alternative approach to the synthesis of 26-iodocholesterol (**7b**) was based on the observation of Brown et al.[5] that trialkylboranes derived from terminal olefins, on exposure to iodine in the presence of base, yield primary iodides. Accordingly, 25-methylenecholesterol (**8a**) was treated with borane and then with iodine and methanolic sodium hydroxide to give, after acid hydrolysis, 26-iodocholesterol (**7b**) in moderate yield.

Electrophilic addition of phenylselenium bromide[6,7] to the 25-olefin **8b**, in the presence of potassium acetate, furnished the 26-phenylseleno 25-acetate **9a**. The NMR of **9a** displayed an AB quartet at δ 3.44 (J_{AB} = 12 Hz), as required for the 26-phenylseleno moiety. A small amount of **9b** (ca. 10%) was also obtained.

The synthesis of the 26-(3-methylindol-1-yl)cholesterol was first modeled on *n*-heptyl bromide. The alkylation of 3-methylindole (**10a**) with *n*-heptyl bromide in the presence of potassium hydroxide in Me_2SO[8] gave *n*-heptyl-3-methylindole (**10b**) in excellent yield. Similar alkylation of (**10a**) with 26-bromocholesterol (**7a**) did not proceed satisfactorily and **11** was obtained in trace amounts only. However, treatment of an excess of the trimethylsilyl ether **7c** with 3-methylindole in Me_2SO–DMF, followed by hydrolysis of silyl ether, gave the desired 26-(3-methylindol-1-yl)cholesterol (**11**) in 50% yield.

Experimental Section

Preparative thin-layer chromatography (TLC) was carried out on silica gel [60 HF 254 + 366) supplied by Merck A. G.

Melting points were taken on a hot stage apparatus and are corrected. Infrared spectra were recorded on a Perkin-Elmer Model 237 spectrophotometer (KBr). Nuclear magnetic resonance (NMR) spectra were recorded in the indicated solvents on a Varian HA-100A spectrometer. Chemical shifts are quoted in parts per million downfield from internal tetramethylsilane (s = singlet, d = doublet, dd = doublet of doublets, m = multiplet). Mass spectra were recorded on a Nuclide Co. Model 12-90-G mass spectrometer equipped with a General Automation SPC 16/45 data acquisition system and a Printronix Model P-300 printer.

16β,26-Dihydroxycholesterol (2, Tetrahydrodiosgenin). Concentrated hydrochloric acid (60 mL) was added dropwise (60 min) to a boiling mixture of diosgenin (1, 2 g), freshly prepared zinc amalgam (60 g), and ethanol (200 mL). Following the addition of the concentrated HCl, the mixture was refluxed for 15 min and filtered. The cooled filtrate was poured, with stirring, into ice–water (1.5 L). The obtained solid was filtered and suspended in

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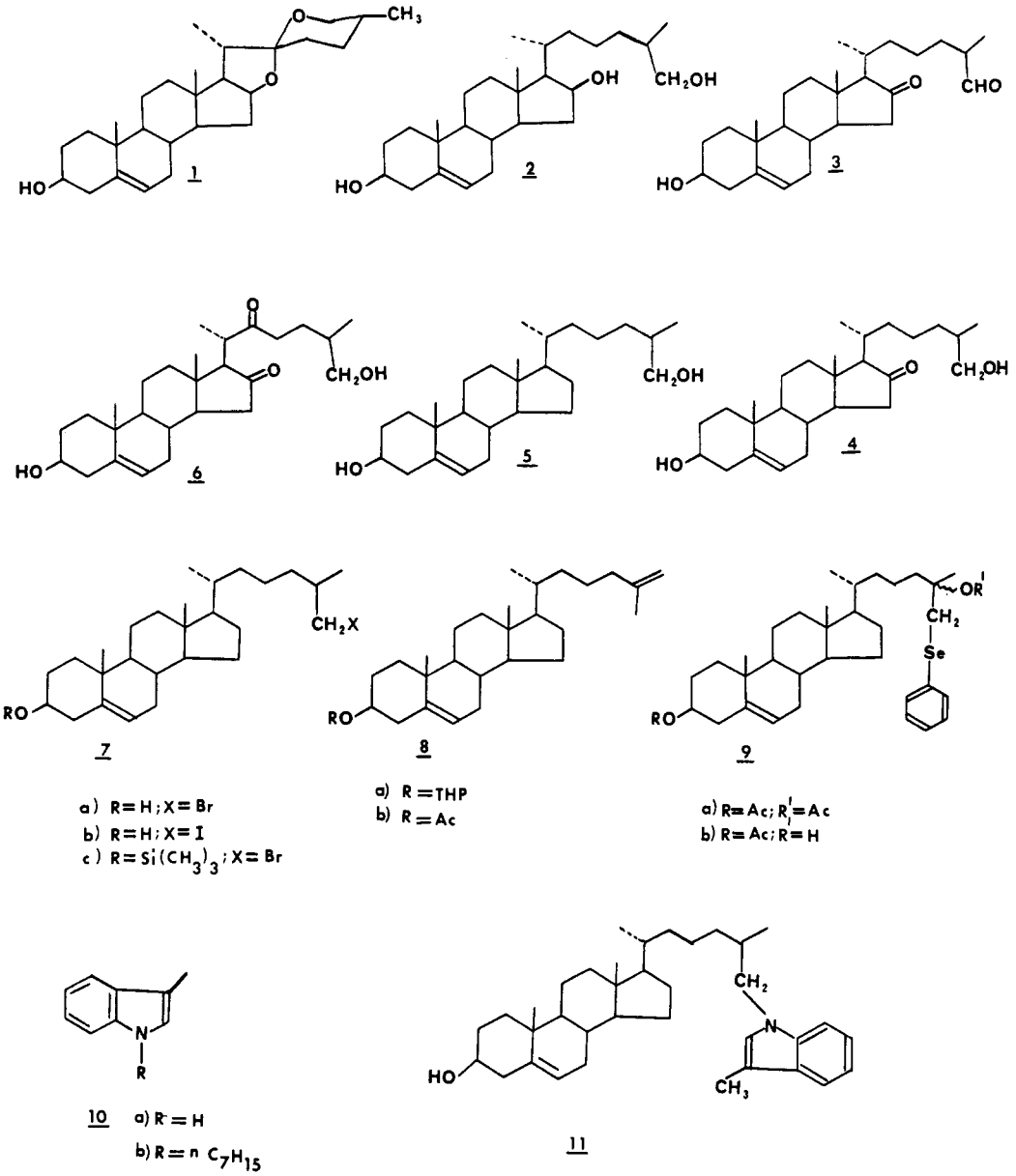
Chart I



ether (15–20 mL), and the mixture was stirred for 5–10 min. The product was filtered and crystallized from ethyl acetate to yield tetrahydrodiosgenin (**2**): 1 g; mp 176–178 °C; NMR ($CDCl_3$ + Me_2SO-d_6) δ 3.43 (2 H, d, J = 6 Hz, CH_2OH), 4.32 (1 H, m, 16α-H), 5.4 (1 H, m, C-6 H).

Oxidation of Tetrahydrodiosgenin (2). A solution of chromium trioxide (80 mg) in water (0.4 mL) and acetic acid (0.8 mL) was added dropwise to a stirred mixture of tetrahydrodiosgenin (**2**, 500 mg) and sodium acetate (2.48 g) in glacial acetic acid (90 mL). The mixture was stirred for 18 h at 25 °C, and then the excess reagent was destroyed with several drops of methanol. Most of the solvent was removed by distillation under reduced pressure at 30–40 °C, and the residue was diluted with cold water. The product was recovered with methylene dichloride, and the extract was washed with water, aqueous sodium bicarbonate, and water, dried (Na_2SO_4), and concentrated. TLC of the fluffy residue (480 mg) revealed the presence of two major products. The mixture was resolved by column chromatography (silica gel, hexane–ethyl acetate). The initial fractions furnished **25-formyl-3β-hydroxycholest-5-en-16-one (3)**: 106 mg; mp 151–154 °C (hexane–ethyl acetate); IR (KBr) 1720 (aldehyde), 1736 (16-ketone) cm^{-1}; NMR ($CDCl_3$) δ 3.7 (m, 3α-H), 5.4 (m, C-6 H), 9.62 (d, J = 2 Hz, HC=O).

Subsequently, the 16-oxo-26-hydroxycholesterol (**4**, 313 mg) was eluted: mp 167–169 °C (from hexane–ethyl acetate); IR (KBr) 1738 cm^{-1} (16-ketone); NMR ($CDCl_3$) δ 3.48 (d, J = 6 Hz, CH_2OH), 3.68 (m, 3α-H), 5.4 (m, C-6 H). Finally, from the more polar eluates the starting triol **2** (35 mg) was obtained.

26-Hydroxycholesterol (5). Method A (from 4). A solution of the 16-ketone (**4**, 1 g), 95% hydrazine (2 mL), and potassium hydroxide (1.35 g) in triethylene glycol (20 mL) was refluxed gently at 150 °C for 30 min. Then the water was slowly distilled until the temperature rose to 195 °C (30 min). The reaction was maintained at this temperature for 2 h, cooled, and poured into 0.5 N hydrochloric acid (200 mL). The solid was filtered, washed (water), and dried. Recrystallization from ethyl acetate furnished cholest-5-ene-3β,26-diol (**5**): 620 mg; fine white needles; mp 175–177 °C; NMR ($CDCl_3$ + Me_2SO-d_6) δ 3.4 (d, J = 6 Hz CH_2OH), 3.68 (m, 3α-H), 5.34 (m, C-6 H).

Method B (from Kryptogenin (6)). Concentrated HCl (43 mL) was added dropwise (2 h) to a refluxing mixture of kryptogenin (**6**, 2.5 g), zinc amalgam (60 g, freshly prepared), concentrated hydrochloric acid (3.5 mL), and 95% ethanol (180 mL). The mixture was refluxed for another 0.5 h, cooled, decanted from the zinc into cold water (1 L), and stored at 10 °C for ca. 15 h. The semicrystalline residue was recovered with ether, the ethereal solution was washed with water, 2% sodium bicarbonate solution, and water and dried (Na_2SO_4), and the solvent was removed. The residue was crystallized from ethyl acetate to give 1.2 g of a 1:1 mixtue of 26-hydroxycholesterol (**5**) and the 16-ketone **4**.

The mixture was reduced by the Wolff–Kishner method, as described in method A, to give pure 26-hydroxycholesterol: 0.9

g; mp 175–178 °C (ethyl acetate).

26-Bromocholesterol (7a). Triphenylphosphine (660 mg, 2.5 mmol) was added in small portions to a cooled (ice bath) and stirred mixture of 26-hydroxycholesterol (500 mg, 1.25 mmol), *N*-bromosuccinimide (450 mg, 2.5 mmol), and DMF (25 mL). The color of the solution changed from pale yellow to orange and back to pale yellow. The solution was stirred at 0–5 °C for 15 min and then at 50 °C for 15 min. Excess reagent was destroyed with methanol and the solvent removed under reduced pressure. Ether was added, and the organic phase was washed with water, dried, and concentrated to yield a white solid. The solid was chromatographed on a column of silica gel, and elution with hexane–ethyl acetate (5:1) gave 26-bromocholesterol (**7a**): 378 mg; mp 134–136 °C (hexane–ethyl acetate); NMR δ 3.32 (d, J = 6 Hz, CH_2Br), 3.64 (3α-H), 5.38 (m, C-6 H); mass spectrum, m/e 466, 464 (M^+ ions).

26-Iodocholesterol (7b). Method A (from Cholest-5-ene-3β,26-diol (5)). To a cooled (ice bath) and stirred solution of 26-hydroxycholesterol (**5**; 500 mg, 1.25 mmol) and *N*-iodosuccinimide (565 mg, 2.5 mmol) in DMF (25 mL) was added triphenylphosphine (660 mg, 2.5 mmol) gradually. The color changed from pale yellow to dark pink, brownish pink, light pink, brown, orange, and then back to pale yellow. The reaction mixture was stirred for 15 min at 0.5 °C and then for 15 min at 50 °C. Excess reagent was destroyed with methanol and the solvent removed under reduced pressure. The residue was diluted with cold water (100 mL), and a 5% sodium thiosulfate solution was added with stirring until the pink color disappeared. The resulting white solid was filtered and dried, and the crude product was chromatographed on a column of silica gel. Elution of the column with hexane–ethyl acetate (5:1) furnished 26-iodocholesterol (**7b**): 339 mg; mp 118–120 °C (MeOH); NMR ($CDCl_3$) δ 3.2 (d, J = 6 Hz, CH_2I), 3.68 (m, 3α-H) 5.4 (m, C-6 H); mass spectrum, m/e 512 (M^+).

Method B (from 25-Methylenecholesterol 3-THP Ether (8a)). A dry two-necked flask equipped with a septum and a venting system was flushed with N_2 and charged with a solution of 25-methylenecholesterol 3-THP ether (**8a**;[9] 234 mg, 0.5 mmol) in dry tetrahydrofuran (THF, 2 mL). Then a 1 M solution of borane in THF (0.183 mL) was injected dropwise, and the mixture was stirred for 1 h at 25 °C. The excess reagent was destroyed with methanol (0.01 mL). Stirring was continued, and iodine (127 mg, 0.5 mmol) was added, followed by dropwise addition of a 3 M solution of sodium hydroxide in methanol (0.168 mL, 5 min). The reaction mixture was stirred for 5 min and then poured into a 2% sodium thiosulfate solution (20 mL). The product was recovered with ether and processed in the conventional manner to yield a residue (220 mg). The residue was dissolved in methanol (10 mL) containing a few drops of concentrated hydrochloric acid, and the solution was stored for 30 min at 25 °C. The solvent was removed, and the residue was worked up in ethyl acetate to yield a semicrystalline solid. Following fractionation by preparative TLC (silica gel; hexane–ethyl acetate, 2:1), 26-iodocholesterol (**7b**, 76 mg) was obtained.

26-(Phenylseleno)cholest-5-ene-3β,25-diol Diacetate (9a). Diphenyl diselenide (80 mg, 0.26 mmol) was added to a stirred solution of bromine (40 mg, 0.25 mmol) in acetic acid (5 mL), and after 15 min, 25-methylenecholesterol acetate (**8b**, 200 mg) and anhydrous potassium acetate (150 mg) were added to the homogeneous red solution of phenylselenium bromide. The red solution first turned brown and then brownish orange. The mixture was stirred (1 h) and diluted with water (50 mL), and the product was extracted with ethyl acetate. The extract was washed with 5% $NaHCO_3$ and water and dried (Na_2SO_4), and the solvent was removed under reduced pressure at 40 °C. The resulting residue was fractionated by preparative TLC (hexane–ethyl acetate (5:1), developed twice). The obtained mixture of (25*R*)- and (25*S*)-selenides **9a** (170 mg) resisted crystallization: NMR δ 0.66 (3 H, s, C-18 H), 1.0 (3 H, s, C-19 H), 1.5 (3 H, s, C-27 H), 1.87 (3 H, s, 25-OAc), 2.01 (3 H, s, 3-OAc), 3.44 (2 H, AB q, J_{AB} = 12 Hz, CH_2SePh), 4.58 (1 H, m, 3α-H), 5.35 (1 H, m, C-6 H), multiplets centered at 7.22 (3 H) and 7.5 (2 H) (5 aromatic protons); mass spectrum, no M^+, m/e 584 (^{80}Se), 582 (^{80}Se), 580 (^{78}Se, M^+ – acetic acid). In addition, **9b** (15 mg) was isolated as a gummy product: NMR δ 0.66 (3 H, s, C-18 H), 1.0 (3 H, s, C-19 H), 1.25 (3 H, s, C-27 H), 2.01 (3 H, s, 3-OAc), 3.13 (2 H, s, CH_2SePh), 4.57 (1 H, m, 3α-H), 5.36 (1 H, C-6 H) and multiplets centered at 7.22 (3 H) and 7.55 (2 H) (5 aromatic protons); mass spectrum, no M^+, m/e 542 (^{82}Se), 540 (^{80}Se), 538 (^{78}Se, M^+ – acetic acid).

***N*-Heptyl-3-methylindole (10b).** A mixture of KOH (2.24 g, 0.04 mol) and dry Me_2SO (20 mL) was stirred at 22–25 °C. After 5 min, 3-methylindole (1.31 g, 0.01 mol) was added and stirring continued (45 min). Then 1-bromoheptane (3.58 g, 0.02 mol) was added dropwise while the temperature of the reaction mixture was maintained at 22-25 °C (external cooling). Stirring was continued for another 1 h, water (150 mL) was added, and the products were recovered with ether. Processing of the ether extract resulted in an oil. GC analysis of the oil indicated the presence of **10b** and 1-bromoheptane; the starting material, 3-methylindole (**10a**), was not detected. The excess bromoheptane was removed by heating the products at 80 °C (0.1 mm) for 1 h. The residue was then dissolved in ether (100 mL) and filtered through a small column of neutral alumina, and the column was washed with ether. The combined ether solution was concentrated to give *n*-heptyl-3-methylindole: 2.2 g; oil; NMR δ 0.8 (3 H, t, J = 6 Hz, CH_2CH_3), 1.3 (10 H, br s, $(CH_2)_5CH_3$), 2.3 (3 H, s, 3-CH_3), 4.01 (2 H, t, J = 6 Hz, $NCH_2(CH_2)_5$), 6.7 (1 H, s, C-2 H), multiplets between 7.1 and 7.5 (4 H, aromatic protons); mass spectrum, m/e 229 (M^+).

26-(3-Methylindol-1-yl)cholest-5-en-3β-ol (11). 26-Bromocholesterol (**7a**, 200 mg) was treated with Sylon BTZ pyridine in dioxane to yield 3-trimethylsilyl ether **7c**. A mixture of KOH (56 mg, 1 mmol) and dry Me_2SO (2 mL) was stirred, and after 5 min, 3-methylindole (33 mg, 0.25 mmol) was added. Stirring was continued for 45 min, and then a solution of silyl ether **7c** (150 mg) in DMF (3 mL) was added dropwise while the mixture was kept at 20 °C. After 1.5 h, 5% HCl (50 mL) was added, and stirring was continued for 15 min. The mixture was made basic with aqueous 1 M NaOH and the product extracted with ethyl acetate. The organic solution was washed, dried, and concentrated to give a gummy residue. Fractionation by preparative TLC yielded homogenous **11** which was recrystallized from methanol: mp 121–124 °C; NMR δ 0.66 (3 H, s, C-18 H), 2.32 (3 H, s, 3′-CH_3), 3.5 (m, 3α-H), 3.86 (2 H, dd, J = 6 Hz, 26-CH_2), 5.35 (1 H, m, C-6 H), 6.8 (1 H, s, 2′-H), multiplets between 7.2 and 7.7 (4 H, aromatic); mass spectrum, m/e 515 (M^+).

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