Chemistry of Natural Compounds, Vol. 36, No. 6, 2000

NOVEL SYNTHESIS OF 5-HYDROXY-5α-CHOLESTA-2,7-DIEN-6-ONE AND ITS CRIEGEE HYDROXYLATION

N. V. Kovganko and S. N. Sokolov

UDC 547.92

*Cholesterol (**1**) is used to synthesize again 5α-hydroxy-2,7-dien-6-one (**5**) through the intermediates **2-4**. cis-Hydroxylation of **5** with OsO_4 and subsequent acetylation give steroids **6-8**. Dehydration of 5α-hydroxy-6-ketone **6** forms the unsaturated compounds **9-11**.*

Key words: cholesterol, ecdysteroids, *cis*-hydroxylation.

Steroidal 5α-hydroxy-2,7-dien-6-ones are important intermediates in the synthesis of insect hormones, ecdysteroids [1-5]. They can be used to prepare ecdysteroids and their analogs that contain a 5α-hydroxy or 4-double bond in their structure. Thus, the synthesis of ecdysteroid analogs through the 5α-hydroxy-2,7-dien-6-ketosteroid **5**, which is prepared rather circuitously from cholesterol and 7-dehydrocholesterol, has been described [1]. Our goal was to develop a method for introducing the 2α,3α-dihydroxy-4,7-dien-6-keto moiety into steroids that is based on **5**. This structural fragment ocurs in ecdysteroids diaulusterols A and B, which are isolated from the open-gilled mollusk *Diaulula sandiegensis* [6]. Our synthetic plan called for *cis*-hydroxylation of the 2-double bond in **5** by OsO_4 by the Criegee reaction with subsequent elimination of the 5α-hydroxy group. Hydroxylation of the 2-double bond in Δ^2-5α-hydroxy-6-ketosteroids by OsO_4 is known to form mainly the corresponding 2α,3α-diols [7,8]. It was also shown that the 2α,3α-diol is formed by the analogous reaction of ergostane $\Delta^{2,7}$-5α-hydroxy-6-ketones [2].

We used the scheme developed earlier in the stigmastane series to synthesize 2,7-dien-5α-hydroxy-6-ketosteroid **5** [4]. Thus, starting cholesterol (**1**) was first reacted with thionyl chloride by the literature method [9] to give 3β-chloroderivative **2** in 95% yield. Treatment of **2** with hydrogen peroxide in a mixture of formic acid and THF with subsequent Jones oxidation by chromic acid gave 3β-chloro-5α-hydroxy-6-ketone **3** in 44% overall yield.

It should be noted that this version of synthesizing **3** is more economical compared with that described previously [10]. Bromination of **3** with heating in acetic acid in the presence of HBr gave 3α-chloro-5α-hydroxy-7α-bromoketone **4** in 60% yield. Dehydrohalogenation of **4** by Li_2CO_3 and LiBr with heating in DMF produced the required 2,7-dien-5-hydroxy-6-ketone **5** in 41% yield. The structures of **2-5** were proved by comparing their spectra with those of the corresponding stigmastane compounds that were synthesized previously (see Experimental) [4].

Two variations were used for Criegee *cis*-hydroxylation of **5** by OsO_4. The first used oxidation by N-methylmorpholine-N-oxide in the presence of a catalytic amount of OsO_4. For the second method, **5** was reacted with an equimolar amount of OsO_4. The triols that were formed in both reactions were not isolated. They were acetylated to give the corresponding acetates. It was found that the same products **6-8** were formed regardless of the method of reacting **5** with OsO_4. Their structures were determined using spectral data. Thus, IR, UV, and ^{1}H NMR spectra indicate that **6-8** contain the Δ^7-6-ketone group. Judging from the presence in the ^{1}H NMR spectrum of multiplets for the methine protons with δ 5.27 and 5.31 ppm, **6** contains a 2,3-diacetate.

The half-widths of these signals (W/2 21 and 9 Hz, respectively) are consistent with an equatorial acetate in the steroid. The other is axial, i.e., the acetates are *cis* to each other.

The analogous conclusion follows from ^{1}H NMR spectra of **8**. The structure of **6** as 2β,3β-diacetoxy-5α-hydroxy-Δ^7-6-ketone was finally proved by the complete agreement of its principal ^{1}H NMR parameters with those of an analogous

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, 220141, Belarus, Minsk, ul. akad. Kuprevicha, 5/2. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 470-473, November-December, 2000. Original article submitted January 9, 2001.

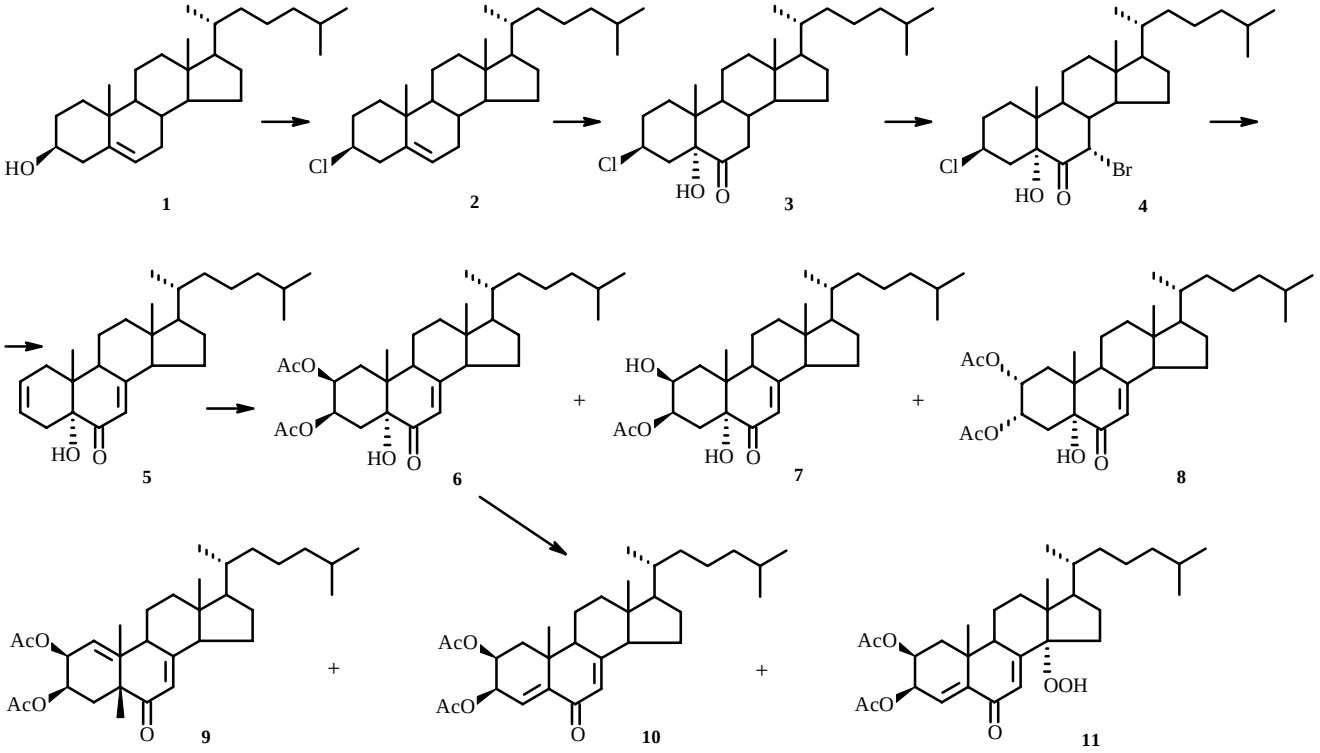
 0009-3130/00/3606-0590$25.00

stigmastane derivative that was obtained by us earlier [5] via Woodward hydroxylation of the corresponding 5α-hydroxy-2,7-dien-6-one. Compounds **6** and **8** are isomers. Therefore, **8** should be assigned the 2α,3α-diacetate structure.

The appearance of multiplets for the methine protons, which are geminal to the hydroxy and acetoxy groups, respectively, at δ 4.15 and 5.20 ppm in the ^{1}H NMR spectrum of **7** is important for proving its structure. Of the two possible structures (i.e., 2β-hydroxy-3β-acetoxy- or 2α-acetoxy-3β-hydroxy-), the first was chosen based on the acetylation of **7** over a long time. Thus, 2β,3β-diacetoxy-5α-hydroxy-Δ^7-6-ketone **6** that is identical to the authentic compound is formed.

Steroid **6** was dehydrated by thionylchloride in pyridine. The principal product of this reaction was the -$\Delta^{4,7}$-6-ketone **10**. However, the two side products **9** and **11** were formed in addition to it. Thus, 1(10),7-dien-6-one **9** is obtained through Westphalen—Lettre backbone rearrangement whereas 14α-hydroperoxide **11** is a typical product of auto-oxidation of **10** by atmospheric oxygen. It should be noted that the formation of 14α-hydroperoxides from Δ^7-ketosteroids has been observed previously [5, 11]. The structures of **9-11** were proved by comparing their spectra with those of the analogous stigmastane derivatives that we obtained from β-sitosterol [5].

We propose that the predominant formation of 2β,3β-diols via *cis*-hydroxylation of 5α-hydroxy-2,7-dien-6-one **5** is due mainly to steric hindrance to the approach of the reagent from the α-side that is created by the 5α-hydroxy group. The role of the 7-double bond is not yet clear. One of the hypotheses is that the presence of a conjugated enone group in the *B* ring of **5** leads to a conformation change in the *A* ring. Examples of such a conformational transition are known in steroid chemistry [12, 13] although they are encountered rather infrequently.



EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra were recorded on a UR-20 instrument in the range 700-3600 cm^{-1} in KBr pellets or films. UV spectra of ethanol solutions were taken on a Specord M-400 instrument. ^{1}H NMR spectra of $CDCl_3$ solutions were obtained on a Bruker AC-200 NMR spectrometer at working frequency 200 MHz. Chemical shifts are given relative to an internal standard of TMS.

3β-Chloro-5-hydroxy-5α-cholestan-6-one (3). A solution of **2** (6.94 g, obtained from **1** by the literature method [9]) in THF (170 mL) and formic acid (40 mL) at 18-20°C was stirred and treated with hydrogen peroxide (17.5 mL, 30%). The reaction temperature was adjusted to 30°C over 3 h. The mixture was stirred for another 2 h and left to cool.

After 19 h the solution was evaporated under vacuum to 3/4 of its volume, treated with water (100 mL), and extracted with CH_2Cl_2 (4×50 mL). The extracts were washed with water (2×60 mL) and evaporated under vacuum. The solid was co-evaporated under vacuum with benzene and then toluene, dried under vacuum, dissolved in ethanolic KOH (300 mL, 5%), and stirred for 1 h. The reaction mixture was neutralized with HCl until the pH was 7, evaporated to 2/3 the volume, treated with water (120 mL), and extracted with CH_2Cl_2 (4×50 mL). The CH_2Cl_2 extracts were washed with water (2×40 mL) and evaporated under vacuum. The solid was co-evaporated with benzene, dried under vacuum, dissolved in acetone (200 mL), and treated with stirring with chromic acid (21 mL, 8 N).

The excess of oxidant was decomposed after 0.5 h by adding isopropanol (30 mL). The reaction mixture was evaporated under vacuum to 3/4 of the volume, diluted with water (120 mL), and extracted with ethylacetate (4×50 mL). The combined extracts were washed with water (2×40 mL) and dried with $MgSO_4$. The solvent was evaporated under vacuum. The solid was chromatographed on an Al_2O_3 column with elution by petroleum ether—ethylacetate of increasing polarity (from 30:1 to 2:1). Yield of **3**, 3.32 g, 44%, mp 182-183°C (ethanol—ethylacetate), lit. [10] mp 180-186°C. IR spectrum (ν, cm^{-1}): 1715 (C=O). ^{1}H NMR spectrum (δ, ppm): 0.65 (18-Me, s), 0.83 (19-Me, s), 0.86 (26-Me, 27-Me, d, J = 6.5 Hz), 0.91 (21-Me, d, J = 6 Hz), 2.70 (H-7α, t, J = 12.5 Hz), 4.21 (H-3α, m, W/2 = 21 Hz).

3β-Chloro-7α-bromo-5-hydroxy-5α-cholestan-6-one (4). A solution of **3** (7.62 g) in glacial acetic acid (250 mL) and HBr (3 mL, 32%) at 45-50°C was stirred and treated with bromine in glacial acetic acid (10 mL, 2 M) over 40 min. It was stirred at the same temperature for 5 h and left to cool. After 17 h the reaction mixture was evaporated under vacuum to ~60 mL volume. Successive crystallizations gave **4**, 5.39 g, 60%, mp 132-135°C (ethanol). Found, %: C 63.29, H 8.50, Br + Cl 22.38. Calc. for $C_{27}H_{44}O_2BrCl$, %: C 62.85, H 8.59, Br + Cl 22.36. IR spectrum (ν, cm^{-1}): 1720 (C=O). ^{1}H NMR spectrum (δ, ppm): 0.69 (18-Me, s), 0.85 (19-Me, s), 0.87 (26-Me, 27-Me, d, J = 6.5 Hz), 0.93 (21-Me, d, J = 6 Hz), 2.30 (H-4α, ddd, J_1 = 14 Hz, J_2 = 4 Hz, J_3 = 1 Hz), 2.64 (5α-OH, s), 4.22 (H-7β, d, J = 4.5 Hz), 4.24 (H-3α, m, W/2 = 29 Hz).

5-Hydroxy-5α-cholesta-2,7-dien-6-one (5). A solution of **4** (1.75 g) in DMF (65 mL) was treated with Li_2CO_3 (2.79 g) and LiBr (0.66 g). The mixture was heated to 135°C, stirred for 6 h, and cooled to room temperature. The precipitate was filtered off. The filtrate was diluted with water (50 mL) and extracted with ethylacetate (4×50 mL). The extracts were dried over $MgSO_4$ and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by hexane—ethylacetate of increasing polarity (from 50:1 to 10:1). Yield of **5**, 0.62 g, 41%. IR spectrum (ν, cm^{-1}): 1680 (C=O), 1630 (C=C). UV spectrum (λ_{max}, nm): 249 (ε 10,000). ^{1}H NMR spectrum (δ, ppm): 0.61 (18-Me, s), 0.87 (26-Me, 27-Me, d, J = 6.5 Hz), 0.88 (19-Me, s), 0.95 (21-Me, d, J = 6 Hz), 5.69 (H-2, H-3, H-7, m).

Criegee *cis*-Hydroxylation of 5-Hydroxy-5α-cholesta-2,7-dien-6-one (5). A. A solution of **5** (0.62 g) in a mixture of THF, *t*-butanol, and water (50 mL, 10:10:1) was treated with N-methylmorpholine-N-oxide monohydrate (0.63 g) and OsO_4 (0.069 g) in a mixture of THF, *t*-butanol, and water (2 mL, 10:10:1). The mixture was stirred for 22 h at room temperature, treated with saturated aqueous $NaHSO_3$ (3 mL), stirred for 45 min, and filtered through a layer of silica gel. The filtrate was treated with water (50 mL) and extracted with ethylacetate (3×40 mL). The combined extracts were washed with water (2×30 mL), evaporated under vacuum and co-evaporated with benzene. The solid was dissolved in acetic anhydride (2 mL) and pyridine (5 mL). The reaction mixture was stored for 48 h and co-evaporated under vacuum with toluene several times. The solid was separated into fractions by preparative TLC on a silica-gel plate with development by a cyclohexane—ethylacetate mixture of increasing polarity (from 3:1 to 2.8:1).

Fraction 1: 2β,3β-Diacetoxy-5-hydroxy-5α-cholest-7-en-6-one (6). Yield 0.12 g, 15%, mp 185-188°C (ether—petroleum ether), lit. [1] mp 192°C (methanol—water). IR spectrum (ν, cm^{-1}): 1755, 1730, 1255, 1240 (AcO), 1690 (C=O), 1630 (C=C). UV spectrum (λ_{max}, nm): 250 (ε 12,000). ^{1}H NMR spectrum (δ, ppm): 0.58 (18-Me, s), 0.87 (26-Me, 27-Me, d, J = 6.5 Hz), 0.98 (21-Me, d, J = 6 Hz), 1.07 (19-Me, s), 2.02 (AcO, s), 2.08 (AcO, s), 5.27 (H-3α, m, W/2 = 21 Hz), 5.31 (H-2α, m, W/2 = 9 Hz), 5.67 (H-7, t, J = 2 Hz).

Fraction 2: 2α,3α-Acetoxy-5-hydroxy-5α-cholest-7-en-6-one (8). Yield 0.12 g, 15%, mp 207-209°C (ether). IR spectrum (ν, cm^{-1}): 1750, 1255, 1240 (AcO), 1680 (C=O), 1630 (C=C). UV spectrum (λ_{max}, nm): 249 (ε 14,000). ^{1}H NMR spectrum (δ, ppm): 0.60 (18-Me, s), 0.87 (26-Me, 27-Me, d, J = 6.5 Hz), 0.94 (21-Me, d, J = 6 Hz), 1.00 (19-Me, s), 2.01 (2α-AcO, s), 2.12 (3α-AcO, s), 3.26 (5α-OH, s), 5.02 (H-2β, ddd, J_1 = 12 Hz, J_2 = 4 Hz, J_3 = 3 Hz), 5.60 (H-3β, m, W/2 = 7.5 Hz), 5.69 (H-7, t, J = 2 Hz).

Fraction 3: 3β-Acetoxy-2β,5-dihydroxy-5α-cholest-7-en-6-one (7). Yield 0.12 g, 16%, mp 183-186°C (cyclohexane—ethylacetate). IR spectrum (ν, cm^{-1}): 1750, 1730, 1275, 1255 (AcO), 1690 (C=O), 1630 (C=C). UV spectrum (λ_{max}, nm): 250 (ε 10,900). ^{1}H NMR spectrum (δ, ppm): 0.60 (18-Me, s), 0.87 (26-Me, 27-Me, d, J = 6.5 Hz), 0.95 (21-Me,

d, J = 6 Hz), 1.14 (19-Me, s), 2.11 (3β-AcO, s), 4.15 (H-2α, m, W/2 = 9.5 Hz), 5.20 (H-3α, m, W/2 = 19 Hz), 5.65 (H-7, t, J = 1.5 Hz).

B. A solution of **5** (0.2 g) in pyridine (5 mL) was treated with OsO_4 (0.135 g) in pyridine (5 mL). After 23 h the reaction mixture was treated with saturated aqueous $NaHSO_3$ (2 mL), shaken periodically over 30 min, treated with water (60 mL), and extracted with $CHCl_3$ (4×20 mL). The $CHCl_3$ extracts were washed with water (2×20 mL), evaporated under vacuum, and co-evaporated with toluene. The solid was dissolved in pyridine (2.5 mL) and acetic anhydride (1 mL). After 5 h the reaction mixture was co-evaporated several times with toluene. The solid was dissolved in ethylacetate and filtered through a layer of silica gel. The filtrate was evaporated under vacuum. The solid was separated into fractions by TLC on a silica-gel plate with development by a cyclohexane—ethylacetate mixture of increasing polarity (from 3:1 to 2.8:1).

Fraction 1: 0.08 g, 30%, 2β,3β-diacetoxy-5-hydroxy-5α-cholest-7-en-6-one (**6**).

Fraction 2: 0.03 g, 12%, 2α,3α-diacetoxy-5-hydroxy-5α-cholest-7-en-6-one (**8**).

Fraction 3: 0.01 g, 4%, 3β-acetoxy-2β,5-dihydroxy-5α-cholest-7-en-6-one (**7**).

Acetylation of 3β-Acetoxy-2β,5-dihydroxy-5α-cholest-7-en-6-one (7). Compound **7** (0.1 g) was dissolved in pyridine (2 mL) and acetic anhydride (1 mL). After 5 h the reaction mixture was co-evaporated several times with toluene under vacuum. The solid was separated by preparative TLC on a silica-gel plate with development by a cyclohexane—ethylacetate mixture (2.8:1). Yield of **6**, 0.029 g, 27%.

Dehydration of 2β,3β-Diacetoxy-5-hydroxy-5α-cholest-7-en-6-one (6). A solution of **6** (0.2 g) in pyridine (5 mL) cooled to 19°C and deoxygenated three times by evacuating and filling with argon was treated with thionylchloride (0.14 mL). After 15 min water (20 mL) was added. The mixture was extracted with ethylacetate (5×10 mL). The extracts were washed with HCl (2 N, 3×10 mL) and water (3×10 mL) and evaporated under vacuum. The solid was co-evaporated with benzene and separated into fractions on a silica-gel column with elution by a cyclohexane—ethylacetate mixture of increasing polarity (from 15:1 to 5:1).

Fraction 1: Amorphous 2β,3β-Diacetoxy-5-methyl-19-nor-5β-cholesta-1(10),7-dien-6-one (9). Yield 0.012 g, 6%. IR spectrum (ν, cm^{-1}): 1755, 1250, 1230 (AcO), 1690, 1670 (C=O), 1650 (C=C). UV spectrum (λ_{max}, nm): 241 (ε 7,700). ^1H NMR spectrum (δ, ppm): 0.66 (18-Me, s), 0.87 (26-Me, 27-Me, d, J = 6.5 Hz), 0.94 (21-Me, d, J = 6 Hz), 1.45 (19-Me, s), 2.05 (AcO, s), 2.08 (AcO, s), 5.06 (H-3α, m, W/2 = 16 Hz), 5.40 (H-2α, t, J = 4.5 Hz), 5.59 (H-1, d, J = 4.5 Hz), 5.70 (H-7, br. s, W/2 = 4 Hz).

Fraction 2: 2β,3β-Diacetoxycholesta-4,7-dien-6-one (10). Yield 0.040 g, 21%, mp 132-135°C (petroleum ether). IR spectrum (ν, cm^{-1}): 1755, 1250, 1235 (AcO), 1680 (C=O), 1645 (C=C). UV spectrum (λ_{max}, nm): 265 (ε 17,800). ^1H NMR spectrum (δ, ppm): 0.64 (18-Me, s), 0.87 (26-Me, 27-Me, d, J = 6.5 Hz), 0.95 (21-Me, d, J = 6 Hz), 1.24 (19-Me, s), 2.06 (AcO, s), 2.08 (AcO, s), 5.23 (H-2α, m, W/2 = 16 Hz), 5.54 (H-3α, t, J = 4 Hz), 5.87 (H-7, t, J = 2 Hz), 6.22 (H-4, d, J = 4 Hz).

Fraction 3: Amorphous 2β,3β-Diacetoxy-14α-hydroperoxycholesta-4,7-dien-6-one (11). Yield 0.042 g, 20%. IR spectrum (ν, cm^{-1}): 1750, 1250, 1240 (AcO), 1680 (C=O), 1640 (C=C). UV spectrum (λ_{max}, nm): 259 (ε 7,200). ^1H NMR spectrum (δ, ppm): 0.78 (18-Me, s), 0.87 (26-Me, 27-Me, d, J = 6.5 Hz), 0.89 (21-Me, d, J = 6 Hz), 1.26 (19-Me, s), 2.05 (AcO, s), 2.07 (AcO, s), 2.81 (H-9α, m, W/2 = 20 Hz), 5.27 (H-2α, m, W/2 = 13 Hz), 5.53 (H-3α, t, J = 4 Hz), 6.05 (H-7, d, J = 2 Hz), 6.23 (H-4, d, J = 4 Hz).

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