Isolation of Polyhydroxysteroids from the Gorgonian *Acabaria undulata*

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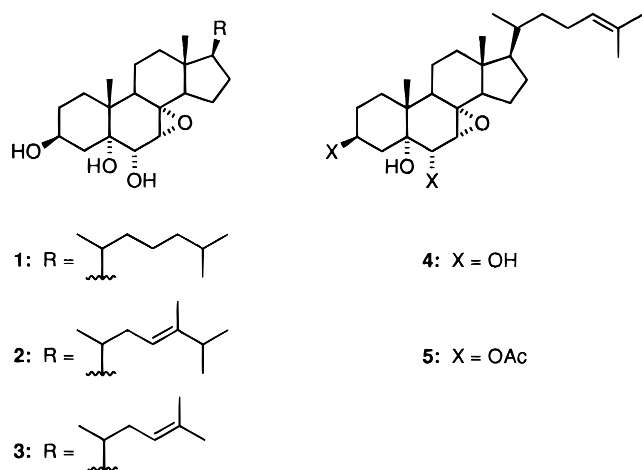
Three steroids and a synthetic diacetoxyl derivative have been isolated from the gorgonian *Acabaria undulata*; the steroids possess a 7α,8α-epoxy-3β,5α,6α-trihydroxyl functionality as a common structural feature. The structures of these compounds were determined by combined spectral methods and chemical transformations. The steroids exhibited moderate cytotoxicity and inhibitory activity against phospholipase A_2.

Recently, we reported the isolation and structure elucidation of four novel ceramides from the gorgonian *Acabaria undulata* Kukental (Melithaeidae, order Gorgonacea) collected along the shore of Keomun Island, Korea.[1] However, ^{1}H-NMR analysis of polar fractions from silica vacuum flash chromatography revealed the presence of several steroids. Because the polarity and ^{1}H-NMR spectra of these metabolites were very different from those of common sterols (e.g., cholesterol), we pursued these compounds. Herein we report the isolation and structure determination of three polyhydroxy steroids (**1**−**3**) and a synthetic diacetate derivative (**5**). All of the compounds possess a 7α,8α-epoxy-3β,5α,6α-trihydroxyl functionality (3β,6α-diacetoxy-7α,8α-epoxy-5α-hydroxyl for **5**) as a common structural feature.

Fractions eluted with polar solvents (80−100% EtOAc in hexane) from silica vacuum flash chromatography were combined and subjected to Sephadex LH-20 column chromatography. Fractions rich in steroids were combined and separated by semi-preparative C_{18} reversed-phase HPLC (60% MeOH in CH_3CN) to yield three compounds (**1**−**3**). Separation of another steroid (**4**), however, was not successful under the HPLC conditions. Subsequently, this compound was purified as its diacetate derivative **5** by acetylation of the mixture followed by C_{18} reversed-phase HPLC.



Compound **1** was isolated as a white solid, and a molecular formula of $C_{27}H_{46}O_4$ was deduced by a combination of HRDEIMS and ^{13}C-NMR spectrometry. The presence of 27 signals in the ^{13}C-NMR spectrum, together with the presence of characteristic methyl proton signals in the ^{1}H-NMR spectrum, revealed that compound **1** was a steroid. The presence of five carbon signals in the region of δ 80−60 in the ^{13}C-NMR spectrum indicated this compound to be highly oxidized. This interpretation was supported by the ^{1}H-NMR spectrum, in which four signals were observed in the region of δ 4.2−3.5. The signals at δ 4.17 (1H, br d, J = 2.3 Hz) and 2.61 (1H, br d, J = 5.4 Hz) disappeared in a D_2O exchange experiment, while the splitting pattern of the proton at δ 3.70 changed from a double doublet (J = 5.4, 0.5 Hz) to a doublet (J = 0.5 Hz) in the same experiment. Thus, compound **1** must possess at least two hydroxyl groups. The presence of an additional hydroxyl group was revealed by the chemical shift and splitting pattern of the proton at δ 3.94 (1H, m), characteristic of the H-3 proton of 3-hydroxysteroids. The functionality of the remaining oxygen was determined by a measurement of $^{1}J_{CH}$ values. A proton-coupled ^{13}C-NMR spectrum showed that $^{1}J_{CH}$ values of the methine carbons at δ 67.81 and 66.55 were 142.8 and 143.3 Hz, respectively, but that the $^{1}J_{CH}$ of the methine carbon at δ 65.52 was 176.1 Hz, characteristic of a strained epoxide ring. Thus, functionalities of all of the oxygens in **1** were determined as an epoxide and one tertiary and two secondary hydroxyl groups.

The structure of **1** was determined by a combination of ^{1}H COSY, HMQC, and HMBC experiments. A long-range correlation between the Me-19 protons at δ 1.02 and a quaternary carbon at δ 77.89 assigned the C-5 hydroxyl group. This interpretation was supported by a correlation between the same carbon and the H-4α proton at δ 2.25 (assigned by a ^{1}H−^{1}H correlation with the H-3 proton). Another correlation between the H-4α and a carbon at δ 67.81 assigned the C-6 hydroxyl group. Finally, the position of the epoxide was assigned to C-7 and C-8 on the basis of a proton coupling between H-6 at δ 3.70 and an epoxide proton at δ 3.60. The structure of the remaining hydrocarbon part was also determined by 2D NMR experiments and a comparison of the ^{13}C-NMR data with known compounds (Table 1).[2,3] Thus, compound **1** was determined as a 7,8-epoxy-3,5,6-trihydroxysteroid of the cholestane class.

Compound **1** possesses five oxygen-bearing asymmetric carbon centers. The large coupling constants ($W_{1/2} \sim 27$ Hz) of the H-3 proton revealed that the C-3 hydroxyl group was β-oriented to the ring A. This

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Table 1. Carbon NMR Assignments for Compounds **1**, **2**, **3**, and **5**[a]

carbon	compound 1	2	3	5
1	32.21	32.21	32.25	31.83
2	30.52	30.50	30.57	26.82
3	66.55	66.53	66.55	69.64
4	38.85	38.85	38.92	35.34
5	77.89	77.90	77.91	76.52
6	67.81	67.80	67.84	70.10
7	65.52	65.50	65.52	62.32
8	67.58	67.55	67.54	66.62
9	40.10	40.11	40.18	39.94
10	40.83	40.85	40.90	41.54
11	22.06	22.07	22.10	21.99
12	38.11	38.02	38.07	37.96
13	42.50	42.37	42.44	42.40
14	53.80	53.87	53.90	53.75
15	21.23	21.09	21.17	21.18
16	27.25	27.64	27.21	27.42
17	55.83	55.55	55.69	55.50
18	12.42	12.60	12.66	12.68
19	18.92	18.91	18.90	19.23
20	35.94	40.85	39.88	40.18
21	18.78	21.24	20.91	20.97
22	35.89	132.55	132.78	137.21
23	28.83	135.20	135.60	127.01
24	39.41	43.03	30.91	41.91
25	27.97	33.14	22.70[b]	28.50
26	22.79	20.12[c]	22.69[b]	22.31[c]
27	22.79	19.62[c]		22.29[c]
28		17.98		
Ac				170.44
				170.23
				21.37
				21.37

[a] NMR spectra were obtained at 125 MHz in $CDCl_3$ solutions. Assignments were aided by DEPT, HMQC and HMBC experiments. [b,c] Signals may be interchanged.

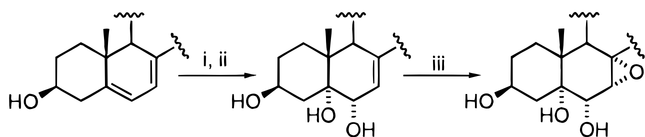

Figure 1. Chemical transformation of 3β-hydroxycholesta-5,7-diene to compound **1**. Key: (i) *m*-CPBA, CH_2Cl_2; (ii) $LiAlH_4$, Et_2O; (iii) *m*-CPBA, Na_2CO_3, CH_2Cl_2, H_2O.

interpretation was supported by a pyridine-induced proton deshielding experiment in which the H-3 proton was shifted downfield by 0.85 ppm.[4] A long-range *W* coupling of the OH-5 proton with the H-4β proton assigned an α orientation for the C-5 hydroxyl group. The α orientation of the C-6 hydroxyl group was determined by a NOESY experiment in which a strong correlation was observed between the H-19 and H-6 protons. Similarly, a strong NOESY correlation between the H-6 and H-7 protons assigned the orientation of the epoxide as α to the B ring. Thus, the structure of compound **1** was determined as 7α,8α-epoxy-3β,5α,6α-trihydroxycholestane. A literature survey revealed that **1** has been synthesized as an oxidation product of 3β-hydroxyergostane.[5-7] To the best of our knowledge, however, this is the first isolation of 7α,8α-epoxy-3β,5α,6α-trihydroxysteroid as a natural product. Comparison of spectral data showed good correlation with published data for this compound.

The structure of **1** was further supported by chemical transformations based upon the method developed by Michand *et al.* (Figure 1).[8] Treatment of 3β-hydroxycholesta-5,7-diene with *m*-CPBA in CH_2Cl_2 followed by reduction with $LiAlH_4$ yielded 3β,5α,6α-trihydroxycholest-7-ene. Epoxidation of this compound with *m*-CPBA in CH_2Cl_2 and water (v/v, 1:1) in the presence of Na_2CO_3 as a catalyst yielded **1**. Spectral data for the synthetic compound were identical with those for the natural one.

Compound **2** was isolated as a white solid, which was analyzed for $C_{28}H_{46}O_4$ by HRDEIMS and ^{13}C-NMR spectrometry. Spectral data for this compound were very similar to those derived from **1**, and the ^{13}C- and ^1H-NMR chemical shifts of the oxygenated positions were almost identical with each other. The only significant difference in NMR data was for signals corresponding to a new double bond and a methyl group. A combination of the ^1H COSY and HMQC experiments readily determined the positions of these groups as Δ^{22} and C-24, respectively. Thus, the structure of **2** was defined as 24-methyl-7α,8α-epoxy-3β,5α,6α-trihydroxycholest-22-ene. Compound **2** possesses a new asymmetric carbon center at C-24. Comparison of the ^1H-NMR data with compounds possessing the identical side chain revealed that the chemical shift of the Me-21 protons (δ 1.021), indicative of the configuration at C-24, was very similar to that of a steroid possessing the 24*R* configuration (δ 1.023).[9] Because the epimeric 24*S* sterol was not isolated from this work, however, the configuration of C-24 is still regarded as tentative.

A related steroid, **3**, was isolated as a white solid that was analyzed for $C_{26}H_{42}O_4$ by HRDEIMS and ^{13}C-NMR spectral data. The spectral data of **3** were very similar to those derived from **1** and **2**. However, only 26 carbon signals were observed in the ^{13}C-NMR spectrum. A combination of ^1H COSY and HMQC experiments readily assigned the position of a double bond to Δ^{22}. The C-23 olefinic proton at δ 5.29 (1H, dd, J = 15.2, 6.8 Hz) was coupled with a proton at δ 2.20 (1H, m) which, in turn, was coupled with a signal corresponding to two methyls at δ 0.95 (6H, d, J = 6.8 Hz). Therefore, compound **3** must be a steroid of the 24-norcholestane class. Steroids possessing this type of truncated side chain have been isolated from sponges as minor metabolites, but the biosynthetic pathway of this side chain has not been investigated.[10]

In addition to compounds **1**–**3**, the ^1H-NMR spectra of the same flash chromatographic fraction revealed the presence of several closely related steroids appearing to differ only in the side chain. Due to their very similar polarities, however, attempts to isolate these compounds were not successful. Subsequently treatment of the inseparable mixture with Ac_2O followed by C_{18} reversed-phase HPLC gave compound **5** as a white solid. The molecular formula of **5** was deduced as $C_{31}H_{48}O_6$ from combined spectral data. Except for changes corresponding to diacetylation at C-3 and C-6, spectral data of **5** were very similar to those of **1**-**3**. A ^1H COSY analysis of the olefinic protons at δ 5.19 (1H, dd, J = 15.1, 8.3 Hz) and 5.31 (1H, dt, J = 15.1, 6.8 Hz) and methyl protons readily determined the structure of **5** as 3β,6α-diacetoxy-7α,8α-epoxy-5α-hydroxycholest-22-ene. Therefore the natural product **4** must be 7α,8α-epoxy-3β,5α,6α-trihydroxycholest-22-ene.

In our measurement of bioactivity, all of the steroids exhibited moderate cytotoxicity against P-388 and DLD-1

Table 2. Cytotoxicity and PLA_2 Inhibitory Effect of Polyhydroxysteroids[a]

compound	P-388	DLD-1	PLA_2
1	17.9	19.6	13.8
2	20.2	22.4	NA[b]
3	28.7	24.6	21.5
5	11.6	12.6	NA[b]

[a] Results are given as IC_{50} values in μM. [b] Not active at the concentration of 100 μM.

cell lines (Table 2). In addition, compounds **1** and **3** exhibited moderate inhibitory activity against phospholipase A_2.

Experimental Section

General Experimental Procedures. NMR spectra were recorded in $CDCl_3$ solutions on a Varian Unity-500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. All of the chemical shifts were recorded with respect to internal TMS. IR spectra were recorded on a Mattson GALAXY spectrophotometer. Mass spectra were obtained by the Mass Spectrometry Facility, Department of Chemistry, University of California, Riverside, by the desorption electron impact mass spectrometry (DEIMS) method. Melting points were measured on a Fisher-Johns apparatus and are reported uncorrected. All solvents used were spectral grade or were distilled from glass prior to use.

Animal Material. *A. undulata* (sample no. 91K-2) was collected by hand using SCUBA at 20−25 m depth in November 1991, along the shore of Keomun Island, South Sea, Korea. The collected samples were briefly dried under shade and kept at −25 °C until chemically investigated.

Extraction and Isolation. The animals (3.9 kg) were defrosted and repeatedly extracted with CH_2Cl_2. The crude extracts (6.5 g) were separated by silica vacuum flash chromatography by using sequential mixtures of *n*-hexane and EtOAc. Fractions eluted with polar solvents (80−100% EtOAc in hexane) were combined and separated by lipophilic Sephadex LH-20 column chromatography (CH_2Cl_2:Me_2CO:EtOH, 2:4:1). Fractions rich in steroids were combined and separated by semi-preparative C_{18} reversed-phase HPLC (YMC ODS column, 1 cm × 25 cm, 60% MeOH in CH_3CN) to obtain compounds **1**−**3** and partially pure **4**.

Compound 1: white solid (42 mg, 0.65% of crude extract); mp 169−171 °C; IR (KBr) ν max 3400, 2960, 2870, 1470, 1380, 1330, 1050, 980, 830 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 4.17 (1H, br d, J = 2.3 Hz, OH-5), 3.94 (1H, m, H-3), 3.70 (1H, dd, J = 5.4, 0.5 Hz, H-6), 3.60 (1H, br s, H-7), 2.61 (1H, br d, J = 5.4 Hz, OH-6), 2.25 (1H, ddd, J = 13.2, 5.0, 2.3 Hz, H-4α), 2.06 (1H, br d, J = 12.1 Hz, H-12β), 1.99 (1H, dd, J = 11.4, 8.2 Hz, H-9), 1.87 (1H, dd, J = 12.8, 6.8 Hz, H-14), 1.84 (1H, m, H-16), 1.82 (1H, m, H-2α), 1.78 (1H, m, H-11α), 1.72 (1H, m, H-11β), 1.65 (1H, m, H-1α), 1.53−1.48 (2H, m, H-1β and H-25), 1.46−1.41 (2H, m, H-4β and H-15α), 1.35−1.31 (4H, m, H-2β, H-20, H-23, and H-23), 1.29−1.24 (3H, m, H-12α, H-16 and H-17), 1.20−1.10 (3H, m, H-15β, H-24, and H-24), 1.02 (3H, s, Me-19), 0.97 (2H, m, H-22), 0.93 (3H, d, J = 6.4 Hz, Me-21), 0.87 (3H, d, J = 6.8 Hz, Me-26/Me-27), 0.86 (3H, d, J = 6.8 Hz, Me-26/Me-27), 0.73 (3H, s, Me-18); HRDEIMS m/z 434.3413 (10), ($C_{27}H_{46}O_4$ requires 434.3396), 416 (12), 398 (14), 277 (7), 265 (100), 128 (20), 95 (15), 81 (16).

Compound 2: white solid (40 mg, 0.62% of crude extract); mp 187−188 °C; IR (KBr) ν max 3400, 2960, 2870, 1460, 1400, 1050, 980, 830 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 5.21 (1H, dd, J = 15.5, 7.7 Hz, H-23), 5.14 (1H, dd, J = 15.5, 8.2 Hz, H-22), 4.18 (1H, d, J = 2.7 Hz, OH-5), 3.95 (1H, m, H-3), 3.70 (1H, br d, J = 2.5 Hz, H-6), 3.60 (1H, br s, H-7), 2.62 (1H, br d, J = 2.5 Hz, OH-6), 2.25 (1H, ddd, J = 13.2, 5.0, 1.8 Hz, H-4α), 2.04 (1H, m, H-12), 2.02 (1H, m, H-20), 2.00 (1H, dd, J = 10.9, 8.1 Hz, H-9), 1.89 (1H, dd, J = 12.8, 7.3 Hz, H-14), 1.85−1.80 (3H, m, H-2α, H-11, and H-24), 1.76−1.70 (2H, m, H-11 and H-16), 1.66 (1H, ddd, J = 13.2, 13.2, 3.2 Hz, H-1α), 1.50 (1H, ddd, J = 13.2, 3.6, 3.6 Hz, H-1β), 1.49−1.40 (3H, m, H-4β, H-15, and H-25), 1.35 (1H, ddd, J = 13.2, 13.2, 4.6 Hz, H-2β), 1.32 (1H, m, H-12), 1.30−1.26 (2H, m, H-16 and H-17), 1.15 (1H, m, H-15), 1.03 (3H, s, Me-19), 1.02 (3H, d, J = 6.8 Hz, Me-21), 0.91 (3H, d, J = 6.8 Hz, Me-28), 0.84 (3H, d, J = 6.8 Hz, Me-26/Me-27), 0.82 (3H, d, J = 6.8 Hz, Me-26/Me-27), 0.74 (3H, s, Me-18); HRDEIMS m/z obsd 446.3396 (12) ($C_{28}H_{46}O_4$ requires 446.3396), 428 (15), 410 (14), 285 (14), 277 (100), 161 (10), 147 (11), 128 (31), 109 (20), 95 (31).

Compound 3: white solid (48 mg, 0.74% of crude extract); mp 184−185 °C; IR (KBr) ν max 3350, 2970, 2870, 1460, 1380, 1050, 970, 830 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 5.29 (1H, dd, J = 15.2, 6.8 Hz), 5.15 (1H, dd, J = 15.2, 8.3 Hz, H-22), 4.19 (1H, d, J = 2.0 Hz, OH-5), 3.94 (1H, m, H-3), 3.69 (1H, br s, H-6), 3.59 (1H, br s, H-7), 2.65 (1H, br s, OH-6), 2.25 (1H, ddd, J = 13.2, 4.9, 2.0 Hz, H-4α), 2.20 (1H, m, H-24), 2.04 (1H, m, H-12), 2.02 (1H, m, H-20), 2.00 (1H, dd, J = 11.0, 8.1 Hz, H-9), 1.89 (1H, dd, J = 12.7, 7.3 Hz, H-14), 1.82 (2H, m, H-2α and H-11), 1.71 (1H, m, H-11), 1.68 (1H, m, H-16), 1.63 (1H, m, H-1α), 1.51 (1H, ddd, J = 13.2, 3.4, 3.4 Hz, H-1β), 1.46 (1H, m, H-4β), 1.43 (1H, m, H-15), 1.36 (1H, m, H-2β), 1.32−1.24 (3H, m, H-12, H-16, and H-17), 1.16 (1H, m, H-15), 1.03 (3H, s, Me-19), 1.02 (3H, d, J = 6.8 Hz, Me-21), 0.95 (6H, d, J = 6.8 Hz, Me-25 and Me-26), 0.75 (3H, s, Me-18); HRDEIMS m/z 418.3077 (8), ($C_{26}H_{42}O_4$ requires 418.3083), 400 (6), 382 (5), 249 (100), 152 (15), 128 (18), 97 (36), 81 (15).

Compound 5. To a stirred solution of 5.1 mg of impure **4** in 0.5 mL of dry pyridine was added 0.3 mL of Ac_2O. After the mixture was stirred for 2 h at room temperature, evaporation under vacuum gave a brown syrup. Separation by semipreparative C_{18} reversed-phase HPLC (YMC ODS column, 100% MeOH) gave 3.2 mg of **5** as a white solid: mp 152−153 °C; IR (KBr) ν max 3450, 2950, 2920, 2860, 1730, 1370, 1235, 1030, 970 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 5.31 (1H, dt, J = 15.1, 6.8 Hz, H-23), 5.19 (1H, dd, J = 15.1, 8.3 Hz, H-22), 5.11 (1H, d, J = 1.0 Hz, H-6), 5.03 (1H, m, H-3), 4.52 (1H, d, J = 2.5 Hz, OH-5), 3.56 (1H, br s, H-7), 2.20 (3H, s, OAc), 2.11 (1H, dd, J = 11.2, 8.3 Hz, H-9), 2.06−1.98 (3H, m, H-12, H-20, and H-4α), 2.00 (3H, s, OAc), 1.95 (1H, m, H-2α), 1.91 (1H, dd, J = 12.7, 7.3 Hz, H-14), 1.89 (1H, m, H-16), 1.82 (2H, m, H-24), 1.79 (1H, m, H-11), 1.76 (1H, ddd, J = 13.4, 3.4, 3.4 Hz, H-1β), 1.72 (2H, m, H-11 and H-16), 1.57 (1H, m, H-25), 1.51 (1H, ddd, J = 12.7, 2.5, 2.5 Hz, H-4β), 1.46 (1H, m, H-1α), 1.41 (1H, m, H-15), 1.39 (1H, m, H-2β), 1.33 (1H,

m, H-12), 1.25 (1H, m, H-17), 1.12 (1H, m, H-15), 1.10 (3H, s, Me-19), 1.03 (3H, d, $J = 6.4$ Hz, Me-21), 0.86 (6H, d, $J = 6.7$ Hz, Me-26 and Me-27), 0.75 (3H, s, Me-18); LRDEIMS m/z 516 (3), 456 (5), 438 (6), 378 (16), 305 (39), 267 (10), 263 (18), 135 (15), 111 (19), 95 (13), 81 (22), 69 (35), 55 (47), 43 (100).

Oxidation of 3β-Hydroxycholesta-5,7-diene. To a stirred solution of 3β-hydroxycholesta-5,7-diene (1.01 g, 2.6 mmol) in dry CH_2Cl_2 (50 mL) was added m-CPBA (0.37 g, 2.2 mmol) and this was stirred for 30 min at room temperature. The reaction mixture was sequentially washed with 10% Na_2CO_3 (50 mL, × 2), 5% $NaHCO_3$ (50 mL × 2), and brine (30 mL). After the solvent was removed under vacuum, the residue was separated by silica vacuum flash chromatography (100% Et_2O). Final purification by semipreparative silica HPLC (YMC silica column, 40% EtOAc in hexane) gave 0.48 g (0.86 mmol) of 6α-(3-chlorobenzoyl)-3β,5α-dihydroxycholest-7-ene: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.03 (1H, dd, $J = 2.0$, 2.0 Hz, H-2′), 7.95 (1H, dd, $J = 7.8$, 2.0 Hz, H-6′), 7.55 (1H, dd, $J = 7.8$, 2.0 Hz, H-4′), 7.39 (1H, dd, $J = 7.8$, 7.8 Hz, H-5′), 5.53 (1H, d, $J = 1.5$ Hz, H-7), 5.00 (1H, d, $J = 1.5$Hz, H-6), 4.02 (1H, m, H-3), 1.07 (3H, s, Me-19), 0.93 (3H, d, $J = 6.4$ Hz, Me-21), 0.86 (3H, d, $J = 6.8$ Hz, Me-26), 0.86 (3H, d, $J = 6.8$ Hz, Me-27), 0.57 (3H, s, Me-18).

Reduction of 6α-(3-Chlorobenzoyl)-3β,5α-dihydroxycholest-7-ene. To a stirred solution of 6α-(3-chlorobenzoyl)-3β,5α-dihydroxycholest-7-ene (101 mg, 0.24 mmol) in dry Et_2O (20 mL) was added $LiAlH_4$ (23 mg, 0.59 mmol). The mixture was stirred under N_2 for 3 h at room temperature. Saturated Na_2SO_4 solution (2 mL) was added to the reaction mixture and stirred for 5 min. Saturated NaCl solution (5 mL) was added and extracted with Et_2O (10 mL × 2). After the solvent was removed under vacuum, the residue was separated by silica vacuum flash chromatography to yield 42 mg of pure 3β,5α,6α-trihydroxycholest-7- ene as a white solid, 0.88 mmol: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 5.02 (1H, br s, H-7), 4.00 (2H, m, H-3 and H-6), 0.97 (3H, s, Me-19), 0.92 (3H, d, $J = 6.4$ Hz, Me-21), 0.87 (3H, d, $J = 6.4$ Hz, Me-26), 0.87 (3H, d, $J = 6.4$ Hz, Me-27), 0.55 (3H, s, Me-18).

Epoxidation of 3β,5α,6α-Trihydroxycholest-7-ene. To a stirred solution of 3β,5α,6α-trihydroxycholest-7-ene (28 mg, 0.06 mmol) in a mixture of CH_2Cl_2 and H_2O (3 mL, v/v, 1:1) were added excess Na_2CO_3 (60 mg, 0.57 mmol) and m-CPBA (75 mg, 0.45 mmol). The mixture was stirred overnight at room temperature. After CH_2Cl_2 (40 mL) was added the reaction mixture was washed with 10% Na_2SO_3 (20 mL × 2), 5% $NaHCO_3$ (20 mL × 2), and brine (30 mL) and dried under vacuum. The residue was separated by C_{18} reversed-phase HPLC (YMC ODS column, 60% MeOH in CH_3-CN) to yield pure **1**, 7.6 mg, 1.7×10^{-5} mol. The ^{1}H- and ^{13}C-NMR data of **1** were identical with those of natural product.

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References and Notes

(1) Shin, J.; Seo, Y. *J. Nat. Prod.* **1995**, *58*, 948−953.
(2) Notaro, G.; Piccialli, V.; Sica, D.; Corriero, G. *J. Nat. Prod.* **1991**, *54*, 1570−1575.
(3) Seo, Y.; Shin, J.; Song, J.-I. *J. Nat. Prod.* **1995**, *58*, 1291−1296.
(4) Kobayashi, M.; Kanda, F.; Rao, C. V. L.; Kumar, S. M. D.; Trimurtulu, G.; Rao, C. B. *Chem. Pharm. Bull.* **1990**, *38*, 1724−1726.
(5) Rodewald, W. J.; Boncza-Tomaszewski, Z. W. *Tetrahedron Lett.* **1979**, 169−172.
(6) Anastasia, M.; Fiecchi, A.; Scala, A. *Tetrahedron Lett.* **1979**, 3323−3326.
(7) Fujimoto, Y.; Miyasaka, S.; Ikeda, T.; Ikekawa, N.; Ohnishi, E.; Mizuno, T.; Watanabe, K. *J. Chem. Soc., Chem. Commun.* **1985**, 10−12.
(8) Michand, D. R.; Nashed, N. T.; Jerina, D. M. *J. Org. Chem.* **1985**, *50*, 1835−1840.
(9) Notaro, G.; Piccialli, V.; Sica, D.; Pronzato, R. *J. Nat. Prod.* **1992**, *55*, 773−779.
(10) Giner, J.-L. *Chem. Rev.* **1993**, *93*, 1735−1753.

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