# Study on Cytotoxic Oxygenated Desmosterols Isolated from the Red Alga *Galaxaura marginata*

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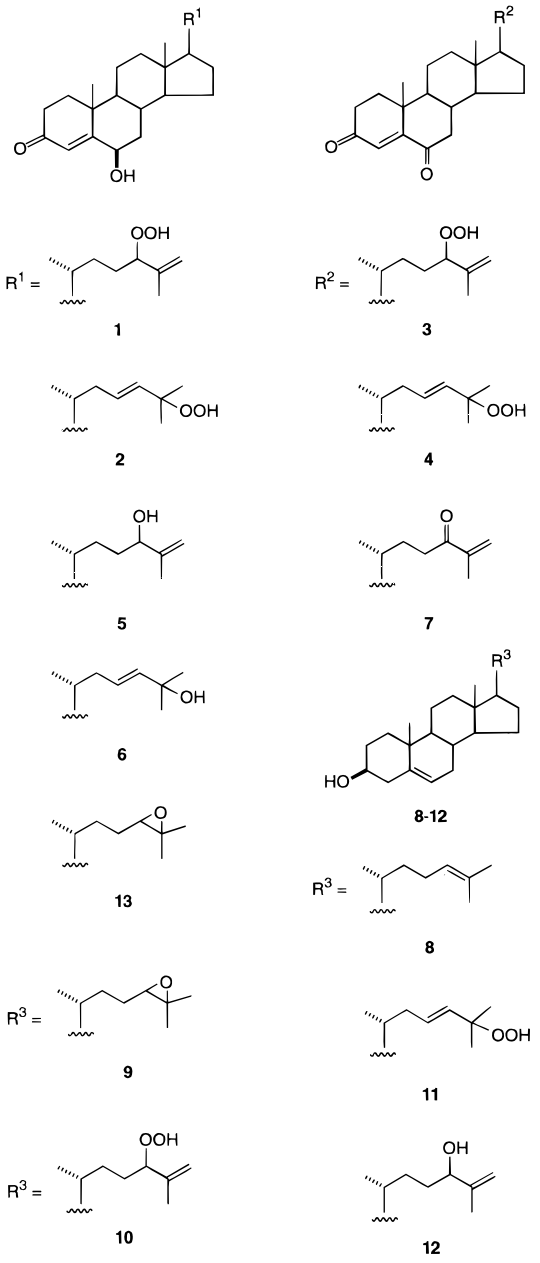
Four novel oxygenated desmosterols, 24ξ-hydroperoxy-6β-hydroxycholesta-4,25-dien-3-one **(1)**, 25-hydroperoxy-6β-hydroxycholesta-4,23(*E*)-dien-3-one (**2**), 24ξ-hydroperoxycholesta-4,25-diene-3,6-dione (**3**), and 25-hydroperoxycholesta-4,23(*E*)-diene-3,6-dione (**4**) were isolated from the marine red alga *Galaxaura marginata*. Steroids **1**−**4** and three synthetic oxygenated desmosterols **5**−**7** were shown to exhibit significant cytotoxicity against several cancer cell lines.

In a search for bioactive metabolites from marine organisms, the red alga *Galaxaura marginata* (Ellis and Solander) Lamouroux, a member of the family Galaxauraceae, was studied because the organic extract of this organism was found to exhibit significant cytotoxicity against KB (human oral epidermoid carcinoma) and P-388 (mouse lymphocytic leukemia) cells. In a previous paper[1] we reported the isolation of desmosterol (**8**) and its oxygenated derivatives 24,25-epoxycholesterol (**9**), 24-hydroperoxycholesta-5,25-dien-3β-ol (**10**), 25-hydroperoxycholesta-5,23(*E*)-dien-3β-ol **(11)**, cholesta-5,25-diene-3β,24-diol (**12**), and 24,25-epoxy-6β-hydroxycholest-4-en-3-one (**13**) from *G. marginata*. Oxygenated desmosterols **10**−**13** were found to be the active principles. Some oxygenated steroids reported previously have also been shown to exhibit cytotoxic properties.[2−5] In this investigation, four additional new cytotoxic steroids, 24ξ-hydroperoxy-6β-hydroxycholesta-4,25-dien-3-one (**1**), 25-hydroperoxy-6β-hydroxycholesta-4,23(*E*)-dien-3-one (**2**), 24ξ-hydroperoxycholesta-4,25-diene-3,6-dione (**3**), and 25-hydroperoxycholesta-4,23(*E*)-diene-3,6-dione **(4)** were obtained from this organism. Steroids **1** and **2** were isolated during a continuation of our study of the hexane-soluble fraction,[1] and compounds **3** and **4** were obtained from a chloroform-soluble fraction of the plant. The structures of these compounds were identified on the basis of spectroscopic methods. This report deals with the isolation, structure determination, and cytotoxicity of these compounds.




## Results and Discussion

Compound **1** was isolated as a white powdery solid. Its molecular formula, $C_{27}H_{42}O_4$, was established by HRMS. The structure of **1** contained a hydroxyl group (IR $\nu_{max}$ 3650 $cm^{-1}$) and a conjugated carbonyl group (IR $\nu_{max}$ 1688 $cm^{-1}$). Its $^1$H-NMR (Table 1) spectral data revealed the presence of two olefinic methylene protons ($H_2$-26) which gave signals as broad singlets at $\delta$ 5.01 and 5.02. A proton geminal with the hydroperoxyl group (H-24) appeared as a triplet at $\delta$ 4.26 ($J$ = 6.8 Hz). A broad singlet at $\delta$ 4.35 was due to H-6, which is geminal with a β-hydroxyl group. A doublet at $\delta$ 0.92 ($J$ = 6.4 Hz) was assigned to the 21-methyl protons. A sharp singlet at $\delta$ 5.82 was due to the olefinic proton, H-4. Two sharp singlets for $CH_3$-18 and $CH_3$-19 appeared at $\delta$ 0.74 and 1.38, respectively. The 27-methyl protons appeared as a singlet at $\delta$ 1.73, indicating that it is on an olefinic double bond. The $^{13}$C-NMR spectral

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**Table 1.** Selective $^1$H-NMR Data of Compounds **1**−**7**[a,b]

| | compound | | | | | | |
|---|---|---|---|---|---|---|---|
| proton | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| H-4 | 5.82 (s) | 5.82 (s) | 6.17 (s) | 6.17 (s) | 5.82 (s) | 5.82 (s) | 6.18 (s) |
| H-6 | 4.35 (br s) | 4.36 (br s) | – | – | 4.35 (br s) | 4.35 (br s) | – |
| H-18 | 0.74 (s) | 0.75 (s) | 0.72 (s) | 0.73 (s) | 0.74 (s) | 0.75 (s) | 0.73 (s) |
| H-19 | 1.38 (s) | 1.38 (s) | 1.16 (s) | 1.16 (s) | 1.38 (s) | 1.38 (s) | 1.16 (s) |
| H-21 | 0.92 (d, 6.4)[b] | 0.92 (d, 6.3) | 0.93 (d, 6.3) | 0.93 (d, 6.3) | 0.94 (d, 6.9) | 0.91 (d, 6.3) | 0.95 (d, 6.6) |
| H-23 | – | 5.65 (dt, 16.2, 7.2) | – | 5.64 (dt, 16.2, 7.2) | – | 5.59 (m) | – |
| H-24 | 4.26 (t, 6.8) | 5.51 (d, 16.2) | 4.27 (t, 6.6) | 5.53 (d, 16.2) | 4.01 (t, 5.7) | 5.59 (m) | – |
| H-26 | 5.01 (s) | 1.34 (s) | 5.02 (s) | 1.34 (s) | 4.84(d, 1.5) | 1.32 (s) | 5.76 (s) |
| | 5.02 (s) | | 5.04 (s) | | 4.93 (d, 1.5) | | 5.95 (s) |
| H-27 | 1.73 (s) | 1.34 (s) | 1.73 (s) | 1.34 (s) | 1.72 (s) | 1.32 (s) | 1.88 (s) |
| OOH | 7.72 (s) | 7.23 (s) | 7.77 (s) | 7.28 | – | – | – |
| | 7.73 (s) | | 7.78 (s) | | | | |

[a] The chemical shifts were determined at 300 MHz in $CDCl_3$. The values are in ppm downfield from TMS. [b] *J* values in hertz in parentheses.

**Table 2.** $^{13}$C-NMR Chemical Shifts of Steroids **1**−**7**[a,b]

| | compound | | | | | | |
|---|---|---|---|---|---|---|---|
| position | **1** | **2** | **3**[c] | **4**[c] | **5** | **6** | **7** |
| C-1 | 37.14 (t) | 37.11 (t) | 35.54 (t) | 35.54 (t) | 37.11 (t) | 37.09 (t) | 35.54 (t) |
| C-2 | 34.28 (t) | 34.26 (t) | 33.97 (t) | 33.97 (t) | 34.26 (t) | 34.24 (t) | 34.35 (t) |
| C-3 | 200.30 (s) | 200.36 (s) | 199.45 (t) | 199.45 (s) | 200.34 (s) | 200.37 (s) | 199.45 (s) |
| C-4 | 126.38 (d) | 126.37 (d) | 125.49 (s) | 125.49 (d) | 126.35 (d) | 126.33 (d) | 125.49 (d) |
| C-5 | 168.30 (s) | 168.30 (s) | 160.97 (s) | 160.97 (s) | 168.33 (s) | 168.36 (s) | 160.98 (s) |
| C-6 | 73.32 (d) | 73.28 (d) | 202.22 (s) | 202.22 (s) | 73.29 (d) | 73.26 (d) | 202.22 (s) |
| C-7 | 38.52 (t) | 38.52 (t) | 46.75 (t) | 46.75 (t) | 38.53 (t) | 38.50 (t) | 46.75 (t) |
| C-8 | 29.70 (d) | 29.73 (d) | 34.19 (d) | 34.19 (d) | 29.73 (d) | 29.71 (d) | 34.19 (d) |
| C-9 | 53.63 (d) | 53.57 (d) | 50.94 (d) | 50.94 (d) | 53.58 (d) | 53.57 (d) | 50.93 (d) |
| C-10 | 38.00 (s) | 37.80 (s) | 39.79 (s) | 39.79 (s) | 37.98 (s) | 37.96 (s) | 39.79 (s) |
| C-11 | 21.00 (t) | 20.96 (t) | 20.86 (t) | 20.86 (t) | 20.97 (t) | 20.94 (t) | 20.87 (t) |
| C-12 | 39.61 (t) | 39.57 (t) | 39.10 (t)$^+$ | 39.05 (t)$^+$ | 39.58 (t) | 39.49 (t) | 39.11 (t) |
| C-13 | 42.55 (s) | 42.57 (s) | 42.56 (s) | 45.56 (s) | 42.53 (s) | 42.53 (s) | 42.59 (s) |
| C-14 | 55.80 (d) | 55.84 (d) | 55.56 (d)$^+$ | 55.65 (d)$^+$ | 55.86 (d) | 55.82 (d) | 55.80 (d) |
| C-15 | 24.13 (t) | 24.16 (t) | 23.91 (t)$^+$ | 23.98 (t)$^+$ | 24.12 (t) | 24.14 (t) | 23.95 (t) |
| C-16 | 28.11 (t) | 28.16 (t) | 27.93 (t)$^+$ | 27.99 (t)$^+$ | 28.14 (t) | 28.12 (t) | 27.93 (t) |
| C-17 | 55.89 (d) | 55.84 (d) | 56.51 (d) | 56.51 (d) | 55.86 (d) | 55.75 (d) | 56.51 (d) |
| C-18 | 12.03 (q) | 12.07 (q) | 11.89 (q)$^+$ | 11.96 (q)$^+$ | 12.02 (q) | 12.05 (q) | 11.92 (q) |
| C-19 | 19.53 (d) | 19.52 (q) | 17.52 (q) | 17.52 (q) | 19.51 (q) | 19.50 (q) | 17.51 (q) |
| C-20 | 35.48 (d) | 35.96 (d) | 35.39 (d) | 35.88 (d) | 35.56 (d) | 36.02 (d) | 35.41 (d) |
| | 35.62 (d) | | | | | | |
| C-21 | 18.61 (q) | 18.69 (q) | 18.56 (q) | 18.67 (q) | 18.69 (q) | 18.61 (q) | 18.49 (q) |
| C-22 | 31.75 (t) | 39.09 (t) | 31.62 (t) | 39.10 (t) | 31.60 (t) | 38.72 (t) | 33.96 (t) |
| | | | 31.67 (t) | | | | |
| C-23 | 27.09 (t) | 130.46 (d) | 27.01 (t) | 130.15 (d) | 28.11 (t) | 125.31 (t) | 30.57 (t) |
| | 27.41 (t) | | 27.33 (t) | | 28.14 (t) | | |
| C-24 | 90.17 (d) | 134.62 (d) | 90.06 (d) | 134.81 (d) | 76.32 (d) | 139.49 (d) | 202.59 (s) |
| | 90.36 (d) | | 90.26 (d) | | 76.39 (d) | | |
| C-25 | 143.65 (s) | 82.30 (s) | 143.52 (s) | 82.26 (s) | 147.47 (s) | 70.74 (d) | 144.59 (s) |
| | 143.80 (s) | | 143.81 (s) | | | | |
| C-26 | 114.17 (t) | 24.37 (q) | 114.22 (t) | 24.37 (q) | 110.36 (t) | 29.90 (q) | 124.29 (t) |
| | | | 114.66 (t) | | 110.89 (t) | | |
| C-27 | 17.23 (q) | 24.37 (q) | 16.94 (q) | 24.37 (q) | 17.25 (q) | 29.90 (q) | 17.73 (q) |
| | | | 17.20 (q) | | 17.60 (q) | | |

[a] The chemical shifts of **2-7** were determined at 75 MHz, and those of **1** were determined at 100 MHz, in $CDCl_3$. The values are in ppm downfield from TMS. [b] Multiplicities were obtained from DEPT experiments. [c] The values were measured from the 1:1 mixture of **3** and **4**. $^+$Interchangeable signals in the same row.

data of **1** (Table 2) showed the presence of 27 carbons, including one carbonyl carbon ($\delta$ 200.30, C-3). The DEPT spectrum indicated the presence of four methyl, 10 methylene, and eight methine carbons. The remaining five signals in the broad-band spectrum were due to the quaternary carbon atoms. Four olefinic carbons (C-4, C-5, C-25, C-26) and two oxygen-bearing methine carbons (C-6, C-24) were further identified. On the basis of all the spectral data, and by comparison of these data with those of 24-hydroperoxycholesta-5,25-dien-3$\beta$-ol **(10)** and 24,25-epoxy-6$\beta$-hydroxycholest-4-en-3-one **(13)**,[1] the structure of **1** was determined as 24-hydroperoxy-6$\beta$-hydroxycholesta-4,25-dien-3-one. The presence of the 6-hydroxy-4-ene-3-carbonyl skeleton of **1** was also supported by a strong absorption at 237 nm (log $\epsilon$ 4.02) in the UV spectrum. A careful analysis of its $^{13}$C-NMR spectra showed that C-20, C-23, C-24, and C-25 all appeared as two closely spaced signals, indicating that sterol **1** was probably an inseparable mixture of 24*R* and 24*S* epimers.[6] This was further confirmed by the $^1$H-NMR spectrum of **1**, which showed the signals of hydroperoxyl protons as two singlets at $\delta$ 7.72 and 7.73, respectively.[7,8]

The new steroid **2** was isolated as a white powdery solid, and its molecular formula was shown to be $C_{27}H_{42}O_4$ by HRMS. Compound **2** also contained a hydroxyl group (IR $\nu_{max}$ 3616 cm$^{-1}$) and a conjugated carbonyl group (IR $\nu_{max}$ 1688 cm$^{-1}$). A strong UV absorption at 237 nm (log $\epsilon$ 4.07) indicated the presence of an $\alpha,\beta$-unsaturated carbonyl group in this compound also. Its $^1$H-NMR spectra revealed a hydroperoxyl proton as a singlet at $\delta$ 7.23.[7,8] Three olefinic methine

protons were observed. A doublet at $\delta$ 5.51 ($J$ = 16.2 Hz) and a doublet of triplets at $\delta$ 5.65 (dt, $J$ = 16.2, 7.2 Hz) were due to the H-24 and H-23 olefinic protons, respectively. The large coupling constant ($J$ = 16.2 Hz) was due to the presence of a trans-disubstituted double bond, located at C-23 and C-24. The 6H singlet at $\delta$ 1.34 was assigned to be the signals of two methyl groups, $CH_3$-26 and $CH_3$-27, attached to an oxygen-bearing quaternary carbon (C-25). The $^1$H-NMR spectral data of the H-4, H-6, $H_3$-18, and $H_3$-19 protons were found to be in full agreement with those of steroid **1**. The $^{13}$C-NMR spectrum of **2** (including DEPT experiments, see Table 2) indicated the presence of five methyl, eight methylene, nine methine, and five quaternary carbons. Further, it revealed the presence of four olefinic carbons (C-4, C-5, C-23, C-24) and two oxygen-bearing carbons (C-6, C-25) in **2**. On the basis of the above data, the structure of **2** was thus established as 25-hydroperoxy-6$\beta$-hydroxycholesta-4,23(*E*)-dien-3-one.

The new steroids **3** and **4** were obtained in small amount (1 mg) as a 1:1 mixture. The $^1$H-NMR spectrum of the mixture revealed the presence of two hydroperoxy groups (two singlets at $\delta$ 7.78 and $\delta$ 7.28)[7,8] and showed that compounds **3** and **4** contained side chains similar to those of **1** and **2**, respectively. The $^1$H-NMR spectrum (Table 1) of the mixture was found to be similar to those of **1** and **2** but showed some significant differences in the signals of the A and B rings. The signal of the 19-methyl protons was shifted upfield to $\delta$ 1.16 (1.38 in **1** and **2**), and the H-4 proton was downfield shifted to $\delta$ 6.18 (5.82 in **1** and **2**), while the H-6 proton signal disappeared. The $^{13}$C-NMR and DEPT spectra (Table 2) revealed that in addition to two isomeric side chains, 19 carbons including two carbonyl carbons (C-3, C-6), two olefinic carbons (C-4, C-5), and two methyl carbons (C-18, C-19) were present in each component of the mixture. On the basis of the above data and the mixture's UV maximum absorption at 251 nm (log $\epsilon$ = 3.97), and by comparison with the spectral data of cholest-4-ene-3,6-dione,[9] compounds **3** and **4** were considered to possess a 4-ene-3,6-dione skeleton, leading to the assignment of the structures of **3** and **4** as 24-hydroperoxycholesta-4,25-diene-3,6-dione and 25-hydroperoxycholesta-4,23(*E*)-diene-3,6-dione, respectively. Steroid **3**, like epoxide **1**, was found to be a mixture of 24*R* and 24*S* epimers[6] by careful analysis of its $^{13}$C-NMR spectrum. This could also be confirmed by the $^1$H-NMR spectrum, which showed the signal of the hydroperoxyl proton as two singlets at $\delta$ 7.77 and 7.78, respectively. Natural 4-ene-3,6-diketo steroids from marine environments have been discovered previously from a sponge.[9]

The origin of oxidized steroids containing a hydroperoxyl group, such as **1**−**4**, has been long questioned,[10] although **1**−**4** may be considered as the naturally sensitized photooxygenation products of desmosterol in the plant. The reaction involves the abstraction of an allylic proton by an activated oxygen, like $^1O_2$, along with migration of the carbon−carbon double bond. Several naturally occurring hydroperoxy steroids have been isolated from tunicates[11] and some plants,[1,2,12] supporting the assumption that these compounds are not artifacts. A natural hydroperoxidation has also been used to rationalize the biogenesis of some hydroperoxycycloartanes.[12] On the basis of the above and our previous observation,[1] it is possible that the allylic hydroperoxides **1**−**4** are formed biosynthetically, even if the possibility that they are artifacts arising from autoxidation cannot be ruled out. As there are different kinds of allylic protons in the side chain of desmosterol, two different types of allylic hydroperoxy steroids were obtained. Also, from a biosynthetic point of view it seems that 6$\beta$-hydroxy-4-ene-3-keto steroids **1** and **2** and 4-ene-3,6-diketo steroids **3** and **4** are probably biogenetically related to desmosterol present in this alga[1] and the corresponding 4-ene-3-keto steroids.

**Table 3.** Cytotoxicity of Steroids **1**−**7**

| | cell lines $ED_{50}$ ($\mu$g/mL) | | | |
|---|---|---|---|---|
| compd | P-388 | KB | A-549 | HT-29 |
| **1** | 0.22 | 0.79 | 0.58 | 0.47 |
| **2** | 0.28 | 0.40 | 1.00 | 0.63 |
| **3**/**4** | 0.53 | 1.15 | 0.88 | 1.48 |
| **5** | 0.19 | 0.83 | 2.37 | 0.30 |
| **6** | 0.14 | 0.45 | 1.54 | 0.55 |
| **7** | 0.11 | 0.24 | 0.27 | 0.30 |

[a] For significant activity of pure compounds, an $ED_{50}$ value of ≤4.0 $\mu$g/mL is required. See Geran *et al.*[14]

Our previous results[1] showed that oxygenated desmosterols **10**−**13** exhibited significant cytotoxicity against several cancer cell lines. Our present study also revealed that steroids **1**−**4** could significantly inhibit the growth of P-388, KB, A-549, and HT-29 cells (Table 3). 6$\beta$,24-Dihydroxycholesta-4,25-dien-3-one **(5)** and 6$\beta$,25-dihydroxycholesta-4,23(*E*)-dien-3-one **(6)**, prepared from the reaction of **1** and **2** with triphenylphosphine, respectively, also were found to exhibit significant cytotoxicity toward the above cancer cells. Cholesta-4,25-diene-3,6,24-trione **(7)**, obtained from the oxidation of **10** by chromic anhydride, was found to be more cytotoxic than the other oxygenated desmosterols described here and in our previous report,[1] presumably due to the presence of an $\alpha,\beta$-unsaturated carbonyl group in the side chain.

## Experimental Section

**General Experimental Procedures.** Melting points were determined using a Fisher-Johns melting points apparatus and were uncorrected. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. Ultraviolet spectra (in ethanol) were recorded on a Hitachi U-3210 UV spectrophotometer, and IR spectra were measured on a Hitachi I-2001 infrared spectrophotometer. The NMR spectra were recorded on a VXR-300/5 FT NMR at 300 MHz for $^1$H and 75 MHz for $^{13}$C or on a Varian Unity Plus 400 MHz FT-NMR for $^1$H and 100 MHz for $^{13}$C, respectively, in $CDCl_3$ using TMS as internal standard, unless otherwise indicated. EIMS spectra were obtained with a VG QUATTRO GC/MS spectrometer. HRMS spectra were recorded on a JMX-HX 110 mass spectrometer. Silica gel 60 (Merck, 230−400 mesh) was used for CC. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.20 mm) were used for analytic TLC.

**Plant Material.** The marine alga *G. marginata* was collected along the coast of Kenting, located in the southernmost tip of Taiwan. A voucher specimen (KTRA-005) was deposited in the Department of Marine Resources, National Sun Yat-Sen University.

**Extraction and Separation**. The marine red alga (12.2 kg fresh wt) was collected in February and March 1994 and was freeze-dried. The dried plant material

(2.72 kg) was minced and extracted exhaustively with EtOAc. The organic extract was evaporated to dryness, and the oily residue (20.20 g) was triturated with hexane. The hexane layer (18.56 g) was found to exhibit cytotoxicity against the P-388 cell line with an $ED_{50}$ of 3.71 $\mu$g/mL and the KB cell line with an $ED_{50}$ of 3.46 $\mu$g/mL. The remaining algal tissue was exhaustively extracted with EtOH, and the extract was evaporated. The residue resulted was subjected to partition between chloroform and water. The chloroform layer was evaporated to yield a residue (11.4 g) which showed cytotoxicity against the P-388 cell line with an $ED_{50}$ of 2.09 and the KB cell line with an $ED_{50}$ of 4.30 $\mu$g/mL. The hexane layer was separated by Si gel column using hexane and hexane−EtOAc mixtures of increasing polarity. The mixture of **1** and **2** (20 mg, 1:1) was eluted with hexane−EtOAc (2:1). Further chromatography of the mixture using hexane−EtOAc−benzene (6:2:1) as eluent yielded pure **1** (1.7 mg), mixtures of **1** and **2** (14.1 mg, 1:1), and pure **2** (1.5 mg). CC of the chloroform layer was also undertaken using hexane and hexane−EtOAc mixtures of increasing polarity. The mixture of **3** and **4** (1.0 mg, 1:1) was isolated from the fraction eluted with hexane−EtOAc (5:1). The mixture was further chromatographed with the elution of hexane−EtOAc (7:1 → 6:1 → 5:1) to yield pure **3** and **4**, both in trace quantities.

**24$\xi$-Hydroperoxy-6$\beta$-hydroxycholesta-4,25-dien-3-one (1):** white powder; $[\alpha]^{28}_D$ +9° (*c* 0.11, $CHCl_3$); mp 166−167 °C; UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 237 (3.92); IR (dry film) $\nu_{max}$ 3560, 1688, 1026 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data in Tables 1 and 2; EIMS *m/z* 430 $[M]^+$ (0.7), 412 (6.0, $M^+ - H_2O$), 285 (18.9), 267 (12.0), 245 (4.4), 227 (11.9), 41 (100); HREIMS *m/z* 430.3082 (calcd for $C_{27}H_{42}O_4$, 430.3085).

**25-Hydroperoxy-6$\beta$-hydroxycholesta-4,23-dien-3-one (2):** white powder; $[\alpha]^{30}_D$ +6° (*c* 0.07, $CHCl_3$); mp 159−160 °C; UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 237 (4.07); IR (dry film) $\nu_{max}$ 3616, 1688, 1034, 1020, 972 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data in Tables 1 and 2; EIMS *m/z* 430 $[M]^+$ (0.4), 412 (2.1, $M^+ - H_2O$), 285 (8.2), 267 (6.1), 245 (1.4), 227 (5.2), 43 (100); HREIMS *m/z* 430.3097 (calcd for $C_{27}H_{42}O_4$, 430.3085).

**24$\xi$-Hydroperoxycholesta-4,25-diene-3,6-dione (3) and 25-hydroperoxycholesta-4,23-diene-3,6-dione (4):** $^1H$ and $^{13}C$ NMR data in Tables 1 and 2; EIMS *m/z* 428 $[M]^+$ (0.9), 410 (10.7, $M^+ - H_2O$), 327 (14.8), 283 (51.8), 243 (15.8), 227 (4.5); HREIMS *m/z* 428.2938 (calcd for $C_{27}H_4O_4$, 428.2928).

**Conversion of Hydroperoxyl Sterols into Hydroxy Sterols.** A stirred solution of a mixture of **1** and **2** (10 mg) in 3 mL of diethyl ether was treated with triphenylphosphine (30 mg) at room temperature for 4 h. The solution was evaporated to dryness and separated by CC with the elution of hexane−EtOAc (3:1 → 2:1) to give steroids **5** (3.1 mg) and **6** (2.9 mg).

**6$\beta$,24$\xi$-Dihydroxycholesta-4,25-dien-3-one (5):** white powder; $[\alpha]^{30}_D$ +10° (*c* 0.19, $CHCl_3$); mp 213−214 °C; UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 237 (4.06); IR (KBr) $\nu_{max}$ 3420, 1680, 1040, 1020 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data in Tables 1 and 2; EIMS *m/z* 414 $[M]^+$ (7.9), 396 (1.9, $M^+ - H_2O$), 285 (10.5), 267 (7.7), 245 (1.1), 227 (3.3), 71 (100); HREIMS *m/z* 414.3146 (calcd for $C_{27}H_{42}O_3$, 414.3134).

**6$\beta$,25-Dihydroxycholesta-4,23-dien-3-one (6):** white powder; $[\alpha]^{28}_D$ +7° (*c* 0.20, $CHCl_3$); mp 185−188 °C; UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 238 (4.14); IR (KBr) $\nu_{max}$ 3456, 1680, 1040, 1020, 970 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data in Tables 1 and 2; EIMS *m/z* 414 $[M]^+$ (4.2), 315 (7.0), 285 (15.6), 267 (8.3), 245 (1.6), 227 (5.9), 43 (100); HREIMS *m/z* 414.3136 (calcd for $C_{27}H_{42}O_3$ , 414.3134).

**Cholesta-4,25-diene-3,6,24-trione (7).** To a gently refluxed solution of 10 mg of 24-hydroperoxycholesta-5,25-dien-3$\beta$-ol (**10**) in diethyl ether (3 mL) was added dropwise a solution of $CrO_3$ (0.1 g) in acetic acid (0.3 mL) and water (0.1 mL) with vigorous stirring. Upon complete addition of the reagent, the reaction mixture was stirred for an additional 6 h under reflux. After the reaction mixture was cooled to room temperature, the excess reagent was destroyed with *i*-PrOH (0.5 mL). Water (2 mL) was added, and the mixture was extracted with ether (10 mL × 3). The combined organic extracts were washed with saturated $NaHCO_3$(aq) (5 mL × 3) and water (10 mL) and dried over anhydrous $Na_2SO_4$. The residue was chromatographed on silica gel using hexane−EtOAc (3:1) to afford **7** (2.9 mg) as white powder: $[\alpha]^{29}_D$ −36° (*c* 0.12, $CHCl_3$); mp 117−118 °C; UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 223 (4.11); $^1H$ and $^{13}C$ NMR data in Tables 1 and 2; EIMS *m/z* 410 $[M]^+$ (47.6), 327 (20.2), 285 (17.2), 267 (3.2), 243 (8.0), 84 (100); HREIMS *m/z* 410.2819 (calcd for $C_{27}H_{38}O_3$, 410.2811).

**Cytotoxicity Testing.** KB and P-388 cells were kindly provided by Prof. J. M. Pezzuto, University of Illinois at Chicago; A-549 (human lung adenocarcinoma) and HT-29 (human colon adenocarcinoma) were purchased from the American Type Culture Collection. Cytotoxicity assays of the tested compounds **1**−**7** were carried out by a modification of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[13] The cultured cells were treated at eight concentrations of pure test compounds ranging from 0.000 64 to 50 $\mu$g/mL. All assays were performed in triplicate. The results were expressed as a percentage, relative to control incubations, and the effective dose required to inhibit cell growth by 50% ($ED_{50}$) was determined.

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