New Cytotoxic Oxygenated Fucosterols from the Brown Alga *Turbinaria conoides*

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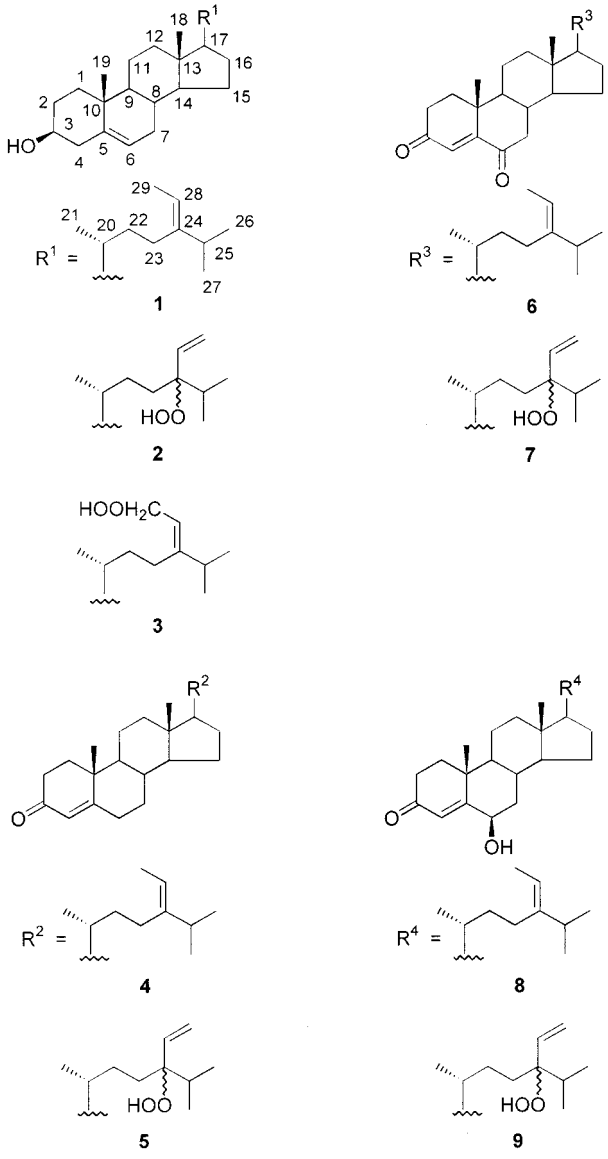
Fucosterol (**1**), 24ξ-hydroperoxy-24-vinylcholesterol (**2**), 29-hydroperoxystigmasta-5,24(28)-dien-3β-ol (**3**), 24-ethylcholesta-4,24(28)-dien-3-one (**4**), 24ξ-hydroperoxy-24-ethylcholesta-4,28(29)-dien-3-one (**5**), 24-ethylcholesta-4,24(28)-dien-3,6-dione (**6**), 24ξ-hydroperoxy-24-ethylcholesta-4,28(29)-dien-3,6-dione (**7**), 6β-hydroxy-24-ethylcholesta-4,24(28)-dien-3-one (**8**), and 24ξ-hydroperoxy-6β-hydroxy-24-ethylcholesta-4,-28(29)-dien-3-one (**9**) were isolated from the marine brown alga *Turbinaria conoides*. The structures of these compounds were established by spectral analysis. Isolated for the first time from a natural source, the oxygenated fucosterols **4**−**9** exhibit cytotoxicity against various cancer cell lines.

The brown algae of the Sargassaceae are abundant seaweeds growing along the coast of southern Taiwan. It has been suggested previously that *Turbinaria* and other members of the family Sargassaceae are inedible due to the inherently great concentrations of polyphenolic substances based upon the polymerization of phloroglucinol.[1,2] Kakisawa and co-workers have reported the isolation of a cytotoxic secosqualene carboxylic acid designated as turbinaric acid from *Turbinaria ornata*.[3] Our previous investigations on the chemical constituents of *Turbinaria conoides* Kützing (Sargassaceae) have led to the isolation of fucosterol **1** and a cytotoxic compound, 24ξ-hydroperoxy-24-vinylcholesterol (**2**).[4] Recently, we also reported the isolation of 29-hydroperoxystigmasta-5,24(28)-dien-3β-ol (**3**), together with **1** and **2** from *T. ornata*.[5] Because some oxygenated steroids have been shown to exhibit cytotoxic properties[5−11] and because the organic extract of *T. conoides* exhibited cytotoxicity against P-388 (mouse lymphocytic leukemia) and KB (human nasopharyngeal carcinoma) cells, we initiated a study to reinvestigate the bioactive substances of this organism.

In the present study, we have isolated the known compounds **1**−**3**, together with six novel steroids: 24-ethylcholesta-4,24(28)-dien-3-one (**4**); 24ξ-hydroperoxy-24-ethylcholesta-4,28(29)-dien-3-one (**5**); 24-ethylcholesta-4,24-(28)-dien-3,6-dione (**6**); 24ξ-hydroperoxy-24-ethylcholesta-4,28(29)-dien-3,6-dione (**7**); 6β-hydroxy-24-ethylcholesta-4,24(28)-dien-3-one (**8**); and 24ξ-hydroperoxy-6β-hydroxy-24-ethylcholesta-4,28(29)-dien-3-one (**9**). This report deals with the isolation, structure determination, and cytotoxic properties of these metabolites.

Results and Discussion

The structures of the known steroids **1**−**3** were established by comparison of their physical and spectroscopic data with those reported previously.[5] The stereochemistry of the double bond at C-24 was determined as *E* in **1** and in the related compounds **4**, **6**, and **8** by the observation of a signal for H-25 at about δ 2.2 ppm in each compound. This proton is known to resonate at about 2.2 ppm in fucosterol and at 2.8 ppm in isofucosterol.[12] The signal of the C-21 methyl protons appeared at about δ 0.99, rather than at 0.95 ppm as in isofucosterol, providing further confirmation for the presence of a trans-double bond at C-24(28) of **1** and the related compounds **4**, **6**, and **8**.[12,13]



Compound **4** was isolated as a white powder. The HRMS of **4** established the molecular formula $C_{29}H_{46}O$, implying

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Table 1. Selected ^{1}H-NMR Data of Steroids **4**−**9**[a]

	compound					
proton	**4**	**5**	**6**	**7**	**8**	**9**
H-4	5.72 (s)	5.73 (s)	6.17 (s)	6.18 (s)	5.82 (s)	5.82 (s)
H-6					4.36 (br s)	4.35 (br s)
H-18	0.72 (s)	0.72 (s)	0.73 (s)	0.73 (s)	0.75 (s)	0.75 (s)
H-19	1.18 (s)	1.18 (s)	1.17 (s)	1.17 (s)	1.38 (s)	1.38 (s)
H-21	0.99 (d, 6.6)[b]	0.96 (d, 6.3)	1.00 (d, 6.6)	0.98 (d, 6.6)	0.99 (d, 6.3)	0.96 (d, 6.9)
H-25	2.22 (m)[c]		2.20 (m)[c]		2.20 (m)[c]	
H-26	0.98 (d, 6.9)	0.86 (d, 6.6)[d]	0.98 (d, 6.9)	0.87 (d, 6.9)[e]	0.98 (d, 6.6)	0.86 (d, 6.9)[f]
H-27	0.98 (d, 6.9)	0.88 (d, 6.6)[d]	0.98 (d, 6.9)	0.89 (d, 6.9)[e]	0.98 (d, 6.6)	0.89 (d, 6.9)[f]
H-28	5.19 (q, 6.6)	5.75 (dd, 17.7, 11.4)	5.19 (q, 6.9)	5.74 (dd, 17.7, 11.4)	5.19 (q, 6.6)	5.75 (dd, 17.4, 11.1)
H-29	1.58 (d, 6.6)	5.16 (dd, 17.7, 1.5)	1,57 (d, 6.9)	5.16 (dd, 17.7, 1.5)	1.57 (d, 6.6)	5.16 (dd, 17.4, 1.5)
		5.28 (dd, 11.4, 1.5)		5.29 (dd, 11.4, 1.5)		5.28 (dd, 11.1, 1.5)
OOH		7.01 (s)		7.01 (s)		7.01 (s)
		7.02 (s)		7.02 (s)		7.02 (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 Hz in parentheses. [c] The chemical shifts were determined by correlations with H_6-26 and -27 in ^{1}H-^{1}H COSY spectra. [d−f] Interchangeable signals in the same column.

Table 2. ^{13}C-NMR Chemical Shifts of Sterols **4**−**9**[a,b]

	compound					
position	**4**	**5**	**6**	**7**	**8**	**9**
C-1	35.7 (t)	35.7 (t)	35.6 (t)	35.6 (t)	37.1 (t)	37.1 (t)
C-2	34.0 (t)	34.0 (t)	34.0 (t)	34.0 (t)	34.3 (t)	34.2 (t)
C-3	199.6 (s)	199.6 (s)	199.5 (s)	199.4 (s)	200.4 (s)	200.5 (s)
C-4	123.8 (d)	123.8 (d)	125.5 (d)	125.5 (d)	126.3 (d)	126.3 (d)
C-5	171.6 (s)	171.6 (s)	161.0 (s)	161.0 (s)	168.4 (s)	168.5 (s)
C-6	33.0 (t)	33.0 (t)	202.3 (s)	202.3 (s)	73.3 (d)	73.3 (d)
C-7	32.1 (t)	32.0 (t)	46.8 (t)	46.8 (t)	38.6 (t)	38.6 (t)
C-8	35.6 (d)	35.6 (d)	34.2 (d)	34.2 (d)	29.7 (d)	29.7 (d)
C-9	53.8 (d)	53.8 (d)	51.0 (d)	51.0 (d)	53.6 (d)	53.6 (d)
C-10	38.6 (s)	38.6 (s)	39.8 (s)	39.8 (s)	38.0 (s)	38.0 (s)
C-11	21.0 (t)	21.0 (t)	20.9 (t)	20.9 (t)	21.0 (t)	21.0 (t)
C-12	39.6 (t)	39.6 (t)	39.1 (t)	39.1 (t)	39.6 (t)	39.6 (t)
C-13	42.4 (s)	42.4 (s)	42.6 (s)	42.6 (s)	42.5 (s)	42.5 (s)
C-14	55.9 (d)	55.9 (d)	55.6 (d)	55.8 (d)	55.8 (d)	55.9 (d)
C-15	24.2 (t)	24.2 (t)	24.0 (t)	24.0 (t)	24.2 (t)	24.1 (t)
C-16	28.2 (t)	28.2 (t)	28.0 (t)	28.0 (t)	28.2 (t)	28.2 (t)
C-17	55.8 (d)	55.9 (d)	56.5 (d)	56.5 (d)	55.9 (d)	55.9 (d)
C-18	11.9 (q)	12.0 (q)	11.9 (q)	11.9 (q)	12.0 (q)	12.0 (q)
C-19	17.4 (q)	17.4 (q)	17.5 (q)	17.5 (q)	19.5 (q)	19.5 (q)
C-20	36.4 (d)	36.2 (d)	36.3 (d)	36.1 (d)	36.4 (d)	36.2 (d)
C-21	18.7 (q)	18.8 (q)	18.7 (q)	18.8 (q)	18.7 (q)	18.8 (q)
C-22	35.2 (t)	28.4 (t)	35.1 (t)	28.3 (t)[c]	35.2 (t)	28.4 (t)[d]
C-23	25.7 (t)	28.4 (t)	25.7 (t)	28.4 (t)[c]	25.7 (t)	28.6 (t)[d]
C-24	146.9 (s)	89.0 (s)	146.8 (s)	89.0 (s)	146.9 (s)	89.1 (s)
C-25	34.8 (d)	31.9 (d)	34.8 (d)	31.9 (d)	34.8 (d)	31.9 (d)
C-26	22.1 (q)	16.7 (q)	22.1 (q)	16.7 (q)	22.1 (q)	16.6 (q)
C-27	22.2 (q)	17.7 (q)	22.2 (q)	18.0 (q)	22.2 (q)	17.7 (q)
C-28	115.6 (d)	137.1 (d)	115.7 (d)	137.1 (d)	115.6 (d)	137.1 (d)
C-29	13.2 (q)	116.4 (t)	13.2 (q)	116.4 (t)	13.2 (q)	116.3 (t)

[a] The chemical shifts were determined at 75 MHz, in $CDCl_3$. The values are in ppm downfield from TMS. [b] Multiplicities were obtained from DEPT experiments. [c,d] Interchangeable signals in the same column.

seven degrees of unsaturation. Its IR spectrum exhibited a conjugated carbonyl stretch at 1680 cm^{-1}, indicating the presence of an α,*β*-unsaturated carbonyl functional group. Its ^{1}H NMR spectral data (Table 1) showed an olefinic proton as a sharp singlet at *δ* 5.72 (H-4) and another olefinic proton as a quartet at *δ* 5.19 (*J* = 6.6 Hz, H-28). Three doublets at *δ* 0.99 (3H, *J* = 6.6 Hz), 0.98 (6H, *J* = 6.9 Hz), and 1.58 (3H, *J* = 6.6 Hz) were due to the H_3-21, H_6-26 and -27, and H_3-29 methyls, respectively. Two sharp singlets for H_3-18 and H_3-19 appeared at *δ* 0.72 and 1.18, respectively. The ^{13}C NMR spectral data (Table 2) of **4** revealed the presence of 29 carbon signals, including one carbonyl carbon (*δ* 199.6, C-3). The DEPT spectrum indicated the presence of six methyl, ten methylene, and eight methine carbons. The remaining five signals in the broad-band spectrum were due to quaternary carbon atoms. Four olefinic carbons (C-4, C-5, C-24, C-28) were further identified. On the basis of all the spectral data, the structure of **4** was established as 24-ethylcholesta-4,24(28)-dien-3-one by comparison of the ^{1}H and ^{13}C NMR data of the side chain with those of fucosterol[5] and by comparison of the corresponding data of the ring system with those of stigmast-4-en-3-one.[14] The presence of the 4-ene-3-carbonyl structural moiety was supported by a strong absorption at 242 nm (log *ϵ* 4.00) in the UV spectrum.

The new steroid **5** was isolated in trace quantities as a white gummy material. The molecular formula $C_{29}H_{46}O_3$ was established by its spectral data, and **5** thus contained seven degrees of unsaturation. Its ^{1}H NMR spectral data (Table 1) showed two olefinic methylene protons (H_2-29), which gave signals at *δ* 5.16 (1H, dd, *J* = 17.7, 1.5 Hz) and 5.28 (1H, dd, *J* = 11.4, 1.5 Hz), and one olefinic methine proton (H-28) appeared at *δ* 5.75 (1H, dd, *J* = 17.7, 11.4 Hz), indicating the presence of a vinyl group in the side chain of the molecule. A sharp singlet at *δ* 5.73 was attributed to the olefinic proton, H-4. Two sharp singlets at *δ* 0.72 and 1.18 were due to the C-18 and C-19 methyl protons, respectively, while a singlet at *δ* 7.01 revealed the presence of a hydroperoxyl proton.

The above ^{1}H NMR data for **5** were found to be similar to those of **4**, but showed some significant differences in side chain signals. The ^{13}C NMR spectrum of **5** (Table 2) indicated the presence of five methyl, eleven methylene, eight methine, and five quaternary carbons, including four olefinic carbons (C-4, C-5, C-28, C-29), one oxygen-bearing carbon (C-24), and a carbonyl carbon (C-3). A careful analysis of its ^{1}H NMR spectrum showed the hydroperoxyl proton signal as two singlets at *δ* 7.01 and 7.02 ppm, indicating that steroid **5** was probably an inseparable mixture of 24*R* and 24*S* epimers.[15,16] However, as only the hydroperoxyl proton appeared as two separated signals in the ^{1}H NMR spectrum and because only a single set of signals appeared in the ^{13}C NMR spectrum, it is also possible that **5** has a single configuration at C-24, the splitting of the hydroperoxyl proton may be due to an unknown factor, rather than to the presence of epimers at C-24. On the basis of the above data, and assuming that the steroid may occur as a mixture of 24*R* and 24*S* epimers, its structure was assigned as 24*ξ*-hydroperoxy-24-ethylcholesta-4,28(29)-dien-3-one (**5**).

Compound **6** was obtained as a pale solid. The molecular formula of **6** was found to be $C_{29}H_{44}O_2$ by HRMS, implying eight degrees of unsaturation. Its IR spectrum showed a conjugated carbonyl stretch at 1694 cm^{-1}. The ^{1}H NMR spectral data (Table 1) of **6** were found to be similar to those of **4**, except that the signal of H-4 was shifted downfield to

δ 6.17 (5.72 in **4**). The ^{13}C NMR and DEPT spectra (Table 2) revealed the presence of 29 carbons, including two carbonyl carbons (C-3, C-6), four olefinic carbons (C-4, C-5, C-24, C-28), and six methyl carbons (C-18, C-19, C-21, C-26, C-27, C-29). On the basis of the above data and the UV maximum absorption at 251 nm (log ϵ 3.83), and by comparison of these data with the spectral data of cholesta-4-ene-3,6-dione,[17] compound **6** was found to be 24-ethylcholesta-4,24(28)-dien-3,6-dione.

Compound **7** was isolated as a pale gummy solid. HRMS established a molecular formula of $C_{29}H_{44}O_4$, and its IR spectrum showed stretching frequencies at 3420 and 1692 cm^{-1}, suggesting the presence of a hydroperoxyl group and a conjugated carbonyl moiety, respectively. The ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) spectral data revealed that steroid **7** has the identical ring structure as that of **6** and the same side chain as that of **5**. Similar to that of **5**, the ^{1}H NMR spectrum of **7** also showed the signal of the hydroperoxyl proton as two closely spaced singlets at δ 7.01 and 7.02 ppm. Thus, compound **7** also was probably an inseparable mixture of 24*R* and 24*S* epimers. On the basis of the above data and the UV maximum absorption at 249.0 nm (log ϵ 3.89), the structure of **7** was then established as 24ξ-hydroperoxy-24-ethylcholesta-4,28(29)-dien-3,6-dione.

Compound **8** was also obtained as a white powder. Its molecular formula, $C_{29}H_{46}O_2$, was established by HRMS, implying seven degrees of unsaturation. Its IR spectrum exhibited stretching frequencies at 3440 and 1680 cm^{-1}, indicating the presence of a hydroxyl group and a conjugated carbonyl group. The ^{1}H NMR spectrum (Table 1) showed that compound **8** contained a side chain similar to those of **4** and **6**. Furthermore, the ^{1}H NMR spectrum of **8** was found to be similar to that of **6,** but showed some significant differences in the signals of the A and B rings. The signal of the C-19 methyl protons was shifted downfield to δ 1.38 (δ 1.16 in **6**), and the H-4 proton was shifted upfield to δ 5.82 (δ 6.17 in **6**), while the H-6 proton signal appeared as a broad singlet at δ 4.36 (disappeared in **6)**. Based on the comparison of the above data with those of 3-oxostigmast-4-en-6β-ol,[14] together with the UV maximum absorption at 239 nm (log ϵ 4.03), the presence of the 6β-hydroxy-4-ene-3-carbonyl skeleton in **8** was confirmed. The ^{13}C NMR spectrum of **8** (including DEPT experiments, see Table 2) indicated the presence of six methyl, nine methylene, nine methine, and five quaternary carbons. Further, it showed that **8** contains four olefinic carbons (C-4, C-5, C-24, C-28), one carbonyl carbon (C-3), and one oxygen-bearing methine carbon (C-6). On the basis of the above data, **8** is 6β-hydroxy-24-ethylcholesta-4,24(28)-dien-3-one.

The new steroid **9** was isolated as a white powder. The HRMS of **9** established the molecular formula $C_{29}H_{46}O_4$, implying seven degrees of unsaturation. A strong UV absorption at 238 nm (log ϵ 4.09) indicated the presence of an α,β-unsaturated carbonyl group in this compound, too. This was further confirmed by its IR spectrum, which exhibited a strong conjugated carbonyl stretch at 1680 cm^{-1}. Its ^{1}H NMR spectrum (Table 1) revealed a hydroperoxyl proton as two singlets at δ 7.01 and 7.02 ppm, respectively. Three olefinic protons of the C-24 vinyl substituent also displayed signals ranging from δ 5.16 to 5.75. Thus, **9** contained the same side chain as that of **5** and of **7**. The chemical shifts of H_3-18, H_3-19, H-4, and H-6 in compound **9** are nearly identical to those of the corresponding protons in compound **8**. Thus, **9** was assumed to be the C-24 hydroperoxylated derivative of **8**. This could be further confirmed by comparing the ^{13}C NMR spectral data of **9** (Table 2) with those of compounds **3**, **5**, and **8**. The structure of **9** was thus established as 24ξ-hydroperoxy-6β-hydroxy-24-ethylcholesta-4,28(29)-dien-3-one.

Table 3. Cytotoxicity of Steroids **4**−**9**[a]

	cell lines ED_{50} (μg/mL)			
compound	P-388	KB	A-549	HT-29
4	>50	>50	>50	>50
6	0.6	5.9	3.1	0.4
7	0.8	4.0	2.5	1.4
8	0.9	4.6	2.3	1.2
9	0.4	1.8	1.8	1.7

[a] For significant activity of pure compounds, an ED_{50} value of $\leq$ 4.0 μg/mL is required. See Geran et al.[19]

The origin of oxidized steroids possessing a hydroperoxyl group has long been questioned.[18] From a biosynthetic point of view it seems that the 4-ene-3-keto steroid **4**; the 4-ene-3,6-diketo steroid **6**; and the 6β-hydroxy-4-ene-3-keto steroid **8** are probably biogenetically derived from fucosterol (**1**) present in this alga. Compounds **4**, **6**, and **8** could be further transformed to the corresponding allylic hydroperoxides **5**, **7**, and **9** via a naturally sensitized photooxygenation, involving the abstraction of an allylic proton by singlet oxygen ($^1O_2$), along with migration of the carbon−carbon double bond. However, the possibility that these hydroperoxides are artifacts arising from the autoxidation of the corresponding steroids cannot be completely ruled out.

The cytotoxicity of compounds **4** and **6**−**9** against the growth of P-388, KB, A-549, and HT-29 cancer cells was studied, and the results are shown in Table 3. These data show that steroid **9** exhibited significant cytotoxicity toward the above four cancer cell lines. Compounds **6**−**8** exhibited significant activity against the growth of P-388, A-549, and HT-29 cancer cells, and moderate cytotoxicity toward KB cells. The biological activity of **5** was not tested, as it was obtained in only trace quantities. Compound **4** was found to be inactive against the above four cancer cell lines, suggesting that further oxidation at C-6 of the 4-ene-3-keto steroids could increase the cytotoxicity of the compounds of this type and may warrant further studies in the future.

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. UV spectra (in EtOH) were recorded on a Hitachi U-3210 UV spectrophotometer, and IR spectra were measured on a Hitachi I-2001 or on a HORIBA FT-720 IR 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, in $CDCl_3$ using TMS as internal standard. EIMS and FABMS were obtained with a VG QUATTRO GC/MS spectrometer. HRMS spectral were recorded on a JMX-HX 110 mass spectrometer. Si gel 60 (Merck, 230−400 mesh) was used for column chromatography. Precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.20 mm) were used for analytic TLC.

Plant Materials. The marine alga *T. conoides* was collected along the coast of Kenting, located in the southernmost tip of Taiwan. A voucher specimen (KTBA 001) was deposited in the Department of Marine Resources, National Sun Yat-Sen University.

Extraction and Separation. The marine brown alga (7.56 kg fresh wt) was collected in January 1996, and freeze-dried. The freeze-dried plant (711.1 g) material was minced and extracted exhaustively with EtOAc. The organic extract was evaporated to dryness, and a dark oily residue (10.5 g) was obtained. The crude extract was found to exhibit cytotoxicity against the P-388 cell line, with an ED_{50} of 0.4 μg/mL, and

against the KB cell line, with an ED_{50} of 6.3 μg/mL. The remaining algal tissue was exhaustively extracted with $CHCl_3$, and the extract was evaporated. The $CHCl_3$ layer (10.27 g) was found to be inactive against the P-388 and KB cell lines. The EtOAc layer was separated by Si gel column chromatography using hexane and hexane−EtOAc mixtures as eluents of increasing polarity. Steroid **4** was eluted with hexane−EtOAc (30:1), **5** and **6** with hexane−EtOAc (20:1); **7** with hexane−EtOAc (15:1); **1** with hexane−EtOAc (5:1); **2**, **3**, and **8** with hexane−EtOAc (3:1); and **9** with hexane−EtOAc (2:1). The structures of compounds **1**−**3** were identified by comparing the physical and spectral data with those of the known compounds.[5]

24-Ethylcholesta-4,24(28)-dien-3-one (4): white powder (5.7 mg); mp 83−84 °C; $[\alpha]^{26}_D$ +52° (*c* 0.24, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 242.0 nm (4.06); IR (dry film) ν_{max} 1680, 1380 cm^{-1}; EIMS *m*/*z* (rel int) 410 (55.9) [M^+], 395 (9.1) [$M^+ - CH_3$], 327 (2.2) [$M^+ - C_6H_{11}$], 313 (90.4) [$M^+ - C_6H_{11} - CH_2$], 271 (12.1) [M^+ − side chain]; HREIMS *m*/*z* 410.3550 (calcd for $C_{29}H_{46}O$ 410.3551).

24ξ-Hydroperoxy-24-ethylcholesta-4,28(29)-dien-3-one (**5**): white gummy solid (0.7 mg); UV (EtOH) λ_{max} (log ϵ) 242.0 nm (4.06); IR (dry film) ν_{max} 3432, 1662, 1382, 1130 cm^{-1}; EIMS *m*/*z* (rel int) 442 (0.6) [M^+], 424 (4.4) [$M^+ - H_2O$], 409 (1.6) [$M^+ - OOH$], 327 (3.3) [$M^+ - C_6H_{10} - OOH$], 313 (27.6) [$M^+ - C_6H_{10} - OOH - CH2$], 271 (22.1) [$M^+$ − side chain].

24-Ethylcholesta-4,24(28)-dien-3,6-dione (**6**): pale solid (4.9 mg); mp 134−136 °C; $[\alpha]^{28}_D$ −39° (*c* 0.24, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 251.2 nm (3.83); IR (CCl_4) ν_{max} 1694, 1390 cm^{-1}; EIMS *m*/*z* (rel int): 424 (9.7) [M^+], 327 (25.8) [$M^+ - C_6H_{11} - CH_2$], 285 (2.8) [M^+ − side chain]; HREIMS *m*/*z* 424.3314 (calcd for $C_{29}H_{44}O_2$ 424.3343).

24ξ-Hydroperoxy-24-ethylcholesta-4,24(28)-dien-3,6-dione (7): pale gummy material (1.6 mg); $[\alpha]^{34}_D$ −9° (*c* 0.075, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 249.0 nm (3.89); IR (CCl_4) ν_{max} 3420, 1692, 1380, 1120 cm^{-1}; FABMS *m*/*z* (rel int) 457 (0.21) [$M^+ + 1$], 439 [$M^+ + 1 - H_2O$], 342 (2.29) [$M^+ + 1 - C_6H_{10} - OOH$], 327 (1.36) [$M^+ - C_6H_{10} - OOH - CH_2$], 285 (0.51) [$M^+$ − side chain]; HREIMS *m*/*z* 456.3268 (calcd for $C_{29}H_{44}O_4$ 456.3241).

6β-Hydroxy-24-ethylcholesta-4,24(28)-dien-3-one (**8**): white powder (1.8 mg); mp 171.5−173 °C; $[\alpha]^{28}_D$ +25° (*c* 0.09, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 239.0 nm (4.03); IR (CCl_4) ν_{max} 3420, 1680, 1380, 1095 cm^{-1}; EIMS *m*/*z* (rel int) 426 (15.2) [M^+], 411 (2.1) [$M^+ - CH_3$], 408 (1.8) [$M^+ - H_2O$], 343 (2.0) [$M^+ - C_6H_{11}$], 329 (58.39) [$M^+ - C_6H_{11} - CH_2$], 287 (38.5) [M^+ − side chain], 269 (28.9) [M^+ − side chain $-H_2O$]; HREIMS *m*/*z* 426.3488 (calcd for $C_{29}H_{46}O_2$ 426.3499).

24ξ-Hydroperoxy-6β-hydroxy-24-ethylcholesta-4,28-(29)-dien-3-one (9): white powdery solid (14.6 mg); mp 151−152 °C; $[\alpha]^{27}_D$ + 16° (*c* 0.34, $CHCl_3$); UV λ_{max} 238.2 nm (log ϵ 4.09, EtOH); IR (CCl_4) ν_{max} 3440, 1680, 1380, 1100 cm^{-1}; FBMS *m*/*z* 459 [$M^+ + 1$]; EIMS *m*/*z* (rel int) 443 (2.0) [$M^+ - CH_3$], 425 (1.2) [$M^+ - OOH$], 329 (4.3) [$M^+ - C_6H_{10} - OOH - CH_2$], 287 (1.0) [$M^+$ − side chain]; HREIMS *m*/*z* 458.3366 (calcd for $C_{29}H_{46}O_4$ 458.3398).

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) cells were purchased from the American Type Culture Collection. The cytotoxic activities of tested compounds against the above four cancer cell lines were assayed with a modification of the MTT [3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide] colorimetric method. For P-388 cells, 200 μL of culture was established at 1500 cells/well in 96-well tissue culture plates (Falcon). Tested compounds were dispensed subsequently to the established culture plate at eight concentrations, ranging from 0.00064 to 50 μg/mL. All assays were performed in triplicate.

To measure the cytotoxic activites of pure compounds or crude fractions against A-549, HT-29, KB, and P-388 cells, each cell line was initiated at 750, 750, 2000, and 1500 cells/well, respectively, in 96-well microtiter plates. Three to eight concentrations encompassing an 8- to 128-fold range were evaluated on each cell line. A-549, HT-29, KB, and P-388 cells were enumerated using MTT after exposure to tested compounds for 6, 6, 3, and 3 days, respectively. To each well, 50 μL of 1 mg/mL MTT was added, and plates were incubated at 37 °C for a further 4 h. Supernatant was aspirated with a Dynatech automatic washer. Formazan crystals were redissolved in DMSO (Merck) for 10 min with shaking, and the plate was read immediately on a microtiter plate reader (Dynatech) at a wavelength of 540 nm. The ED_{50} value was defined as the concentration of test compound resulting in a 50% reduction of absorbance compared to untreated cells in the MTT assay.[20]

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