# Cytotoxic Constituents of the Stems of *Cinnamomum subavenium*

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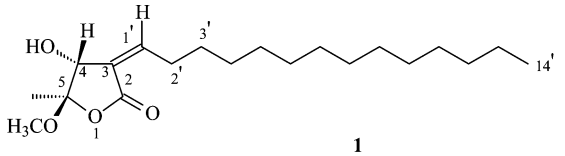
*Received August 28, 2006*

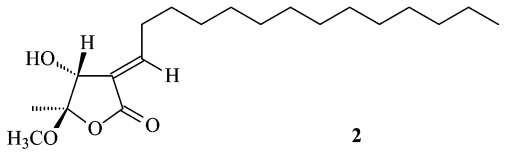
The aim of this study was to evaluate the cytotoxic effects of three new butanolides, subamolides A−C (**1**−**3**), and a new secobutanolide, secosubamolide (**4**), on the human colorectal cancer cell line SW480. Compounds **1**−**4** are new and were isolated from the stems of *Cinnamomum subavenium*, along with 17 known compounds. The structures of **1**−**4** were determined by spectroscopic analysis. Propidium iodide staining and flow cytometry were used to evaluate DNA damage of the treated SW480 cells, and it was found that **1**−**4** caused DNA damage in a dose-dependent manner after 24 h of treatment.

*Cinnamomum subavenium* Miq. (Lauraceae) is a medium-sized evergreen tree, found in central to southern mainland China, Burma, Cambodia, Taiwan, Malaysia, and Indonesia.[1] The chemical constituents and the biological activity of this plant have not yet been reported. In continuation of a program toward the studies of chemotaxonomy and biologically active components from Formosan Lauraceous plants,[2−5] three new butanolides, namely, subamolide A [(3*Z*,4*R*,5*R*)-3-tetradecylidene-4-hydroxy-5-methoxy-5-methylbutanolide] (**1**), subamolide B [(3*E*,4*R*,5*R*)-3-tetradecylidene-4-hydroxy-5-methoxy-5-methylbutanolide] (**2**), and subamolide C [3-(1-methoxypentadecyl)-5-methylene-5*H*-furan-2-one] (**3**), and a new secobutanolide, secosubamolide {methyl[(2*E*)-2-[(1*S*)-1-hydroxy-2-oxopropyl]hexadec-2-enoate]} (**4**), were isolated. Also confirmed were 17 known compounds, including the butanolides linderanolide B[6] and isolinderanolide B,[6] the flavan-3-ols (+)-catechin[7] and (−)-epicatechin,[8] the lignans (−)-sesamin[9] and (+)-syringaresinol,[10] five benzenoids, vanillin,[11] vanillic acid,[12] ferulic acid,[13] *p*-hydroxybenzaldehyde,[14] and syringaldehyde,[15] four steroids, $\beta$-sitosterol,[16] stigmasterol,[16] $\beta$-sitosteryl-D-glucoside,[17] and stigmasteryl-D-glucoside,[17] and two aliphatic compounds, palmitic acid[18] and stearic acid,[19] isolated from the stem of *C. subanenium* by means of MeOH extraction. We report the structural elucidation of the newly isolated butanolides and secobutanolide as well as their effects on DNA damage on a human colorectal cancer cell line, SW480.

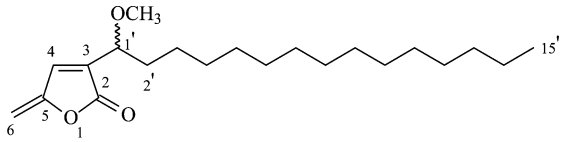
Subamolide A (**1**) was isolated as a colorless oil. Its molecular formula, $C_{20}H_{36}O_4$, was established by HRFABMS. The UV absorption of this compound at 218 nm was similar to that of actinolide B, indicating the presence of an α-alkylidene-saturated $\gamma$-lactone.[20] The IR spectrum of this compound showed absorption bands for a hydroxy group at 3450 $cm^{-1}$ and an $\alpha\beta$-unsaturated $\gamma$-lactone moiety at 1750 and 1680 $cm^{-1}$. The $^1$H NMR spectrum of this compound showed signals for an olefinic proton at $\delta$ 4.39 and an oxygenated methylene at $\delta$ 6.56 (1H, td, $J$ = 8.0, 1.6 Hz, H-1′), while the $^{13}$C NMR spectrum exhibited 20 resonance peaks, including a lactone carbonyl carbon at $\delta$ 167.3, olefinic carbons at $\delta$ 150.2 and 128.9, and a methyl group at $\delta$ 14.1. These spectroscopic features were similar to those of a known lactone, linderanolide B.[6] On comparing with linderanolide B, compound



**1**



**2**



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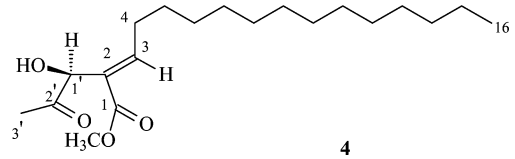

**4**

**1** has an oxygen-bearing quaternary carbon at $\delta$ 108.8, a methoxyl at $\delta$ 50.4, and a methyl carbon at $\delta$ 16.3 but lacked a pair of methylene carbons at $\delta$ 90.3 (C-5) and 157.6 (C-6) in the $^{13}$C NMR spectrum. It could be deduced that compound **1** has methoxyl and methyl groups at C-5 in place of a methylene. The position of these groups was established from the HMBC spectrum (Figure 1). The methyl signal at $\delta_H$ 1.54 was correlated with an oxygen-bearing quaternary carbon at $\delta_C$ 108.8 (C-5) and a methine carbon at $\delta_C$ 75.7 (C-4). The former carbon also correlated with the signal at $\delta_H$ 4.39 (H-4). Furthermore, the methoxyl signal at $\delta_H$ 3.39 also correlated with the C-5 carbon signal $\delta_C$ 108.8 (Figure 1). The geometry of the alkylidene side chain of this compound was *cis* to the carbonyl group on the basis of the chemical shifts of H-1′ ($\delta$ 6.56) and H-2′ ($\delta$ 2.74) in the $^1$H NMR spectrum.[2] This *cis* geometry was confirmed in the NOESY spectrum, which showed cross-peaks between H-4 and H-1′ (Figure 2). The configuration at C-4 in **1** was determined as 4*R* on the basis of the correlations between the $[\alpha]_D$ value [+33.4 (*c* 0.05, $CHCl_3$)] and the configuration at C-4 for 3-alkylidene-4-hydroxyl-5-methylbutanolide derivatives.[21−23]

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10.1021/np060425k CCC: $37.00 
Published on Web 12/01/2006

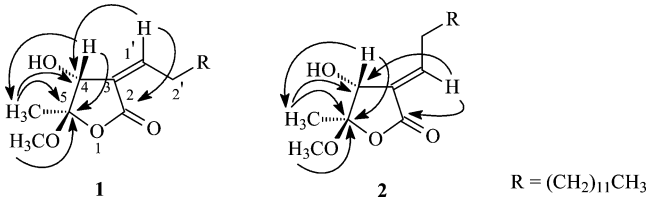


**Figure 1.** HMBC correlations of subamolide A (**1**) and subamolide B (**2**).

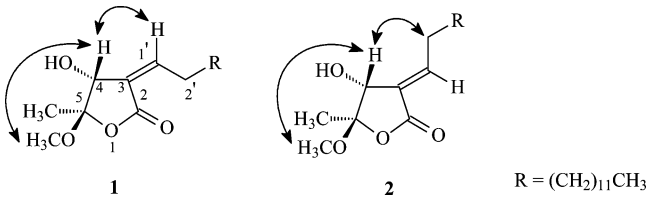


**Figure 2.** NOESY correlations of subamolide A (**1**) and subamolide B (**2**).

**Table 1.** $^1$H NMR Data of Subamolides A (**1**) and B (**2**) (400 MHz, $\delta$ in ppm, $J$ in Hz, $CDCl_3$)

| proton | subamolide A (**1**) | subamolide B (**2**) |
|---|---|---|
| 4 | 4.39 (1H, d, $J = 1.2$) | 4.50 (1H, d, $J = 0.4$) |
| 1′ | 6.56 (1H, td, $J = 8.0, 1.6$) | 6.95 (1H, td, $J = 8.0, 1.6$) |
| 2′ | 2.74 (2H, m) | 2.37 (2H, m) |
| 3′ | 1.49 (2H, m) | 1.50 (2H, m) |
| 4′−13′ | 1.27 (20H, br s) | 1.24 (20H, br s) |
| 14′ | 0.87 (3H, t, $J = 7.0$) | 0.87 (3H, t, $J = 7.0$) |
| OMe-5 | 3.39 (3H, s) | 3.36 (3H, s) |
| Me-5 | 1.54 (3H, s) | 1.59 (3H, s) |

The stereochemistry of C-5 was determined as 5*R* from the correlation between $OCH_3$-5 and the H-4 signal in the NOESY spectrum (Figure 2), indicating that OH-4 and $CH_3$-5 are in the *cis* position.[24] Thus, the structure of compound **1** (subamolide A) was elucidated as (3*Z*,4*R*,5*R*)-3-tetradecylidene-4-hydroxy-5-methoxy-5-methylbutanolide.

Subamolide B (**2**), also a colorless oil, was assigned the molecular formula $C_{20}H_{36}O_4$, as deduced by HRFABMS. Its spectroscopic data (IR, UV, $^1$H and $^{13}$C NMR) were similar to those of subamolide A (**1**). The difference between these compounds involved H-1′, $\delta$ 6.95 (td, $J = 8.0, 1.6$ Hz) in **2** versus $\delta$ 6.56 in **1**, suggesting an *E*-configuration for $\Delta^{3(1')}$ in compound **2**. The $^1$H NMR spectrum of **2** was similar to that of (2*E*,3*R*,4*R*)-2-(11-dodecenylidene)-3-hydroxy-4-methoxy-4-methylbutanolide,[24] indicating that **2** has the same α-alkylidene-saturated γ-lactone skeleton and the same *E* geometry of the trisubstituted double bond [$\delta$ 6.95 (1H, td, $J = 8.0, 1.6$ Hz, H-1′)]. The presence of a broad singlet, $\delta$ 1.24 (20H, br s, H-4′-13′), was attributed to protons in an aliphatic chain in **2**. The geometry of the alkylidene side chain was *trans* to the carbonyl group on the basis of the chemical shifts of H-1′ ($\delta$ 6.95) and H-2′ ($\delta$ 2.37) in the $^1$H NMR spectrum. The downfield shift of H-1′ ($\delta$ 6.95), compared with that of H-1′ ($\delta$ 6.56) in subamolide A (**1**), can be ascribed to the effect of the carbonyl group of a lactone ring. The position of these groups was established from the HMBC spectrum (Figure 1). Its *trans* geometry was also confirmed in the NOESY spectrum, which showed cross-peaks between H-4/H-2′ (Figure 2). The stereochemistry of C-4 and C-5 of **2** was similar to that of subamolide A (**1**).[21−24] Thus, the structure of compound **2** (subamolide B) was elucidated as (3*E*,4*R*,5*R*)-3-tetradecylidene-4-hydroxy-5-methoxy-5-methylbutanolide. The $^1$H and $^{13}$C NMR data of subamolide B (**2**) were assigned by comparing with the data of subamolide A (**1**) (Tables 1 and 2).

Subamolide C (**3**), a colorless oil, was assigned the molecular formula $C_{21}H_{36}O_3$ by EIMS ($[M]^+$, *m*/*z* 336) and HREIMS. The presence of an α,β-unsaturated γ-lactone moiety was apparent from the UV absorption at 265 nm.[2,3] The IR spectrum showed an α,β-unsaturated γ-lactone at 1780 and 1680 cm$^{-1}$.[2,3] The $^1$H NMR spectrum of this compound exhibited the presence of an exomethylene group at $\delta$ 4.88 (1H, d, $J = 2.6$ Hz) and 5.20 (1H, d, $J = 2.6$ Hz), as well as an olefinic proton at $\delta$ 7.21 (1H, br s). Also, it showed signals corresponding to a methoxy functionality at $\delta$ 3.34 (3H, s), an oxymethine proton at $\delta$ 4.12 (1H, dd, $J = 7.4, 4.8$ Hz), and long-chain aliphatic protons at $\delta$ 1.26 (22H, br s) and 1.42−1.68 (4H, m). The structure of this compound was found to be similar to that of the known butanolide 3-(1-methoxynonadecyl)-5-methylene-5*H*-furan-2-one.[2] Thus, the structure of compound **3** (subamolide C) was elucidated as 3-(1-methoxypentadecyl)-5-methylene-5*H*-furan-2-one. Subamolide C (**3**) has a positive specific rotation, but the configuration at C-1′ remained undefined.

**Table 2.** $^{13}$C NMR Data of Subamolides A (**1**) and B (**2**) (100 MHz, $\delta$ in ppm, $CDCl_3$)

| proton | subamolide A (**1**) | subamolide B (**2**) |
|---|---|---|
| 2 | 167.3 (s) | 169.4 (s) |
| 3 | 128.9 (s) | 130.0 (s) |
| 4 | 75.7 (d) | 72.4 (d) |
| 5 | 108.8 (s) | 109.7 (s) |
| 1′ | 150.2 (d) | 148.5 (d) |
| 2′ | 31.9 (t) | 31.9 (t) |
| 3′−13′ | 22.7−29.7 (t) | 22.6−29.8 (t) |
| 14′ | 14.1 (q) | 14.1 (q) |
| OMe-5 | 50.4 (q) | 50.2 (q) |
| Me-5 | 16.3 (q) | 16.0 (q) |

Secosubamolide (**4**), a faint yellowish liquid, also had a molecular formula of $C_{20}H_{36}O_4$, as deduced from HRFABMS. The UV absorption at 215 nm was similar to that of secokotomolide A,[2,3] indicating the presence of a secobutanolide skeleton.[2,3] The IR spectrum of **4** showed characteristic absorption bands due to the presence of hydroxyl (3450 cm$^{-1}$), ester (1735 cm$^{-1}$), and ketone (1710 cm$^{-1}$) groups. The $^1$H NMR spectrum of compound **4** was similar to that of secomahubanolide,[25] with a substitution for the *E* geometry of the trisubstituted double bond [$\delta$ 7.08 (1H, t, $J = 7.6$ Hz, H-3)] in **4** as compared to the *Z* geometry [$\delta$ 6.34 (1H, t, $J = 7.6$ Hz, H-3)] in secomahubanolide.[25] Secomahubanolide (**4**) showed four more methylene units [$\delta$ 1.27 (28H, br s, H-6−19)] than **4** [$\delta$ 1.25 (20H, br s, H-6−15)] in the side chain. An acetyl and one *O*-methyl group were observed at $\delta$ 2.15 (3H, s, H-3′) and 3.73 (3H, s, OMe-1), respectively. Compound **4** showed a positive specific rotation {$[\alpha]^{25}_D$ +42.5 (*c* 0.15, $CHCl_3$)}, indicating the 1′*S* configuration similar to that of secoisolancifolide {$[\alpha]^{25}_D$ +102.7 (*c* 0.49, $CHCl_3$)},[25] but contrary to that of secokotomolide A {$[\alpha]^{25}_D$ −52.1 (*c* 0.15, $CHCl_3$)}.[2] From the above data, compound **4** (secosubamolide) was defined structurally as methyl[(2*E*)-2-[(1*S*)-1-hydroxy-2-oxopropyl]hexadec-2-enoate].

In the present study, we also investigated the effects on DNA of 0 (untreated), 25, 50, 75, and 100 μM of compounds **1**−**4**, after 24 h of treatment, on the human colorectal cancer line SW480, by propidium iodide staining followed by flow cytometry. $SubG_1$ can be considered as a marker for DNA damage, and the appearance of this peak is related to the presence of apoptosis. As shown in Figure 3, untreated cells expressed less than 2.2% of $SubG_1$. DNA damage did not significantly increase by treatment with **1**−**4** at a concentration of 25 μM, and the $SubG_1$ expression was 6.1−8.5%. At a concentration of 50 μM, subamolides A (**1**) and B (**2**) induced significant DNA damage, and $SubG_1$ expression was increased to 25.4% and 23.7%, respectively. The $SubG_1$ levels in subamolide C (**3**) and secosubamolide (**4**) treated cells were less than in **1** and **2** at 50 μM and expressed 11.0% and 9.1% of $SubG_1$, respectively. It is worth noting that DNA damage was significantly increased by all compounds at a concentration of 75 μM. $SubG_1$ levels were increased up to 23.4%−47.2%. Subamolide B (**2**) exhibited the greatest effect on DNA damage at this concentration. At a 100 μM concentration, all compounds induced significant damage to DNA. The order of potency in inducing DNA damage was observed to

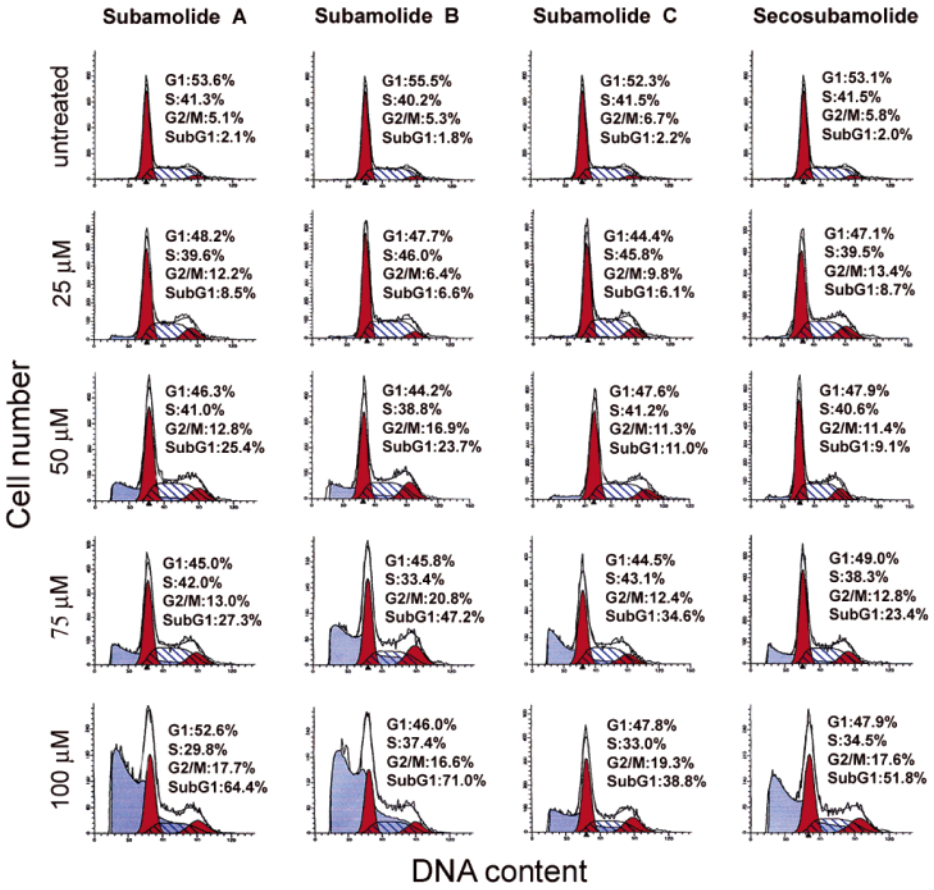


**Figure 3.** Effect of subamolide A (**1**), subamolide B (**2**), subamolide C (**3**), and secosubamolide (**4**) on the cell cycle of the SW480 cell line. SW480 cells were treated with the indicated concentrations of **1**−**4** for 24 h. After treatment, cells were collected, fixed with methanol, stained with propidium iodide, and analyzed by flow cytometry. Data on each sample represent the percentages of $G_1$, S, $G_2$/M, and $SubG_1$, respectively. Analyses were performed at least three times, and a representative experiment is presented.

be as follows: subamolide B (**2**) > subamolide A (**1**) > secosubamolide (**4**) > subamolide C (**3**).

## Experimental Section

**General Experimental Procedures.** Melting points were determined using a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were obtained in MeCN using a JASCO V-530 spectrophotometer. The IR spectra were measured on a Hitachi 260−30 spectrophotometer. $^1$H (400 MHz, using $CDCl_3$ as solvent for measurement), $^{13}$C (100 MHz), DEPT, HETCOR, COSY, NOESY, and HMBC NMR spectra were obtained on a Unity Plus Varian NMR spectrometer. LRFABMS and LREIMS were obtained with a JEOL JMS-SX/SX 102A mass spectrometer or a Quattro GC-MS spectrometer with a direct inlet system. HRFABMS and HREIMS were measured on a JEOL JMS-HX 110 mass spectrometer. Silica gel 60 (Merck, 230−400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.20 mm) were used for analytical TLC, and precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.50 mm) were used for preparative TLC. Spots were detected by spraying with 50% $H_2SO_4$ and then heating on a hot plate. Flow cytometry analysis was performed using a Becton-Dickinson FACS-Calibur flow cytometer. The labeling dye propidium iodide (PI) was used to investigate DNA damage and the cell cycle.

**Plant Material.** The stems of *C. subavenium* were collected from Wulai Hsiang, Taipei County, Taiwan, in May 2005. A voucher specimen (Cinnamo. 5) was characterized by one of the authors (Y.-R.H.) and deposited in the Basic Medical Science Education Center, Fooyin University, Kaohsiung County, Taiwan.

**Extraction and Isolation.** The air-dried stems of *C. subavenium* (8.0 kg) were extracted with MeOH (80 L × 6) at room temperature, and a MeOH extract (202.5 g) was obtained upon concentration under reduced pressure. The MeOH extract, suspended in $H_2O$ (1 L), was partitioned with $CHCl_3$ (2 L × 5) to give fractions soluble in $CHCl_3$ (123.5 g) and $H_2O$ (74.1 g). The $CHCl_3$-soluble fraction (123.5 g) was chromatographed over silica gel (800 g, 70−230 mesh) using *n*-hexane−EtOAc−MeOH mixtures as eluents to produce five fractions. Part of fraction 1 (7.46 g) was subjected to silica gel chromatography by eluting with *n*-hexane−EtOAc (30:1), enriched with EtOAc, to furnish 10 fractions (1-1−1-10). Fraction 1-1 (2.05 g) was subjected to further silica gel chromatography, eluting with *n*-hexane−EtOAc (100:1) and enriched gradually with EtOAc, to obtain four fractions (1-1-1−1-1-4). Fraction 1-1-1 (0.41 g) was further purified by passage over another silica gel column using *n*-hexane−EtOAc mixtures to obtain stearic acid (11 mg) and palmitic acid (42 mg). Fraction 1-1-2 (0.77 g), eluted with *n*-hexane−EtOAc (30:1), was further separated using silica gel column chromatography and preparative TLC (*n*-hexane−EtOAc (30:1) and gave subamolide C (**3**) (15 mg) and secosubamolide (**4**) (221 mg). Fraction 1-1-3 (0.25 g) was subjected to silica gel column chromatography and purified by preparative TLC (thin layer chromatography) to yield vanillin (11 mg) and vanillic acid (16 mg). Fraction 1-3 (4.02 g) was subjected to silica gel chromatography, eluting with *n*-hexane−EtOAc (40:1) and enriched gradually with EtOAc, to obtain three fractions (1-3-1−1-3-3). Fraction 1-3-2 (4.11 g), eluted with *n*-hexane−EtOAc (40:1), was further separated using silica gel column chromatography and preparative TLC (*n*-hexane−EtOAc (30:1) and gave isolinderanolide B (2.31 g) and linderanolide B (134 mg). Part of fraction 2 (9.31 g) was subjected to silica gel chromatography, by eluting with *n*-hexane−EtOAc (10:1), then enriched gradually with EtOAc, to furnish five fractions (2-1-2−5). Fraction 2-1 (2.01 g) was subjected to silica gel chromatography, eluting with $CHCl_3$−MeOH (100:1), and enriched gradually with MeOH, to obtain five fractions (2-1-1−2-1-5). Fractions 2-1-2 (0.21 g) and 2-1-3 (0.21 g) were subjected to further silica gel column chromatography and purified by preparative TLC to yield ferulic acid (9 mg), *p*-hydroxybenzaldehyde (7 mg), and syringaldehyde (10 mg). Fraction 2-4 (1.31 g) was subjected to silica gel chromatography, eluting with *n*-hexane−EtOAc (40:1),

and enriched gradually with EtOAc, to obtain four fractions (2-4-1−2-4-4). Fraction 2-4-2 (1.06 g), eluted with *n*-hexane−EtOAc (10:1), was further separated using silica gel column chromatography and preparative TLC (*n*-hexane−EtOAc (30:1) and gave subamolides A (**1**) (38 mg) and B (**2**) (42 mg). Fraction 2-5 (6.03 g, *n*-hexane−EtOAc (10:1)) was purified by passage over a silica gel column (400 g, 230−400 mesh), using $CHCl_3$−MeOH, to obtain a mixture of $\beta$-sitosterol and stigmasterol (2.26 g). Part of fraction 3 (6.88 g) was subjected to silica gel chromatography, by eluting with *n*-hexane−EtOAc (1:1), enriched gradually with EtOAc, to furnish five fractions (3-1−3-5). Fraction 3-2 (2.89 g) was further purified on a silica gel column using $CHCl_3$−MeOH mixtures to obtain sesamin (45 mg) and syringaresinol (21 mg). Part of fraction 4 (2.33 g) was subjected to silica gel chromatography by eluting with *n*-hexane−EtOAc (1:5), enriched with EtOAc, to furnish five further fractions (4-1−4-5). Fraction 4-2 (1.33 g) was purified on a silica gel column (200 g, 230−400 mesh) using $CHCl_3$−MeOH mixtures to obtain (+)-catechin (37 mg) and (−)-epicatechin (25 mg). A mixture of $\beta$-sitosteryl-D-glucoside and stigmasteryl-D-glucoside (165 mg) was recrystallized (MeOH) from fraction 5. The known compounds have been characterized by comparison of their spectroscopic data with literature values.[6−19]

**Subamolide A [(3*Z*,4*R*,5*R*)-3-tetradecylidene-4-hydroxy-5-methoxy-5-methylbutanolide] (1):** colorless oil; $[\alpha]^{25}_D$ +33.4 (*c* 0.05, $CHCl_3$); UV $\lambda_{max}$ (MeCN) (log $\epsilon$) 218 (4.05) nm; IR (neat) $\nu_{max}$ 3450 (br, OH), 1750, 1680 ($\alpha,\beta$-unsaturated $\gamma$-lactone) $cm^{-1}$; $^1H$ NMR data, see Table 1; $^{13}C$ NMR data, see Table 2; FABMS *m*/*z* 341 $[M + H]^+$ (7), 313 (5), 281 (6), 265 (4), 239 (4), 215 (7), 202 (7), 189 (11), 178 (10), 165 (15), 152 (12), 141 (13), 133 (65), 128 (29), 115 (35), 105 (30), 95 (30), 91 (95), 77 (100), 67 (100); HRFABMS *m*/*z* 341.2693 $[M + H]^+$ (calcd for $C_{20}H_{37}O_4$, 341.2692).

**Subamolide B [(3*E*,4*R*,5*R*)-3-tetradecylidene-4-hydroxy-5-methoxy-5-methylbutanolide] (2):** colorless oil; $[\alpha]^{25}_D$ +37.6 (*c* 0.05, $CHCl_3$); UV $\lambda_{max}$ (MeCN) (log $\epsilon$) 218 (4.02) nm; IR (neat) $\nu_{max}$ 3450 (br, OH), 1750, 1680 ($\alpha,\beta$-unsaturated $\gamma$-lactone) $cm^{-1}$; $^1H$ NMR data, see Table 1; $^{13}C$ NMR data, see Table 2; FABMS *m*/*z* 341 $[M + H]^+$ (6), 313 (5), 281 (4), 265 (4), 239 (5), 215 (6), 202 (7), 189 (10), 178 (13), 165 (15), 141 (13), 133 (24), 128 (15), 115 (24), 105 (32), 95 (42), 91 (82), 79 (87), 69 (100); HRFABMS *m*/*z* 341.2694 $[M + H]^+$ (calcd for $C_{20}H_{37}O_4$, 341.2692).

**Subamolide C [3-(1-methoxypentadecyl)-5-methylene-5*H*-furan-2-one] (3):** colorless oil; $[\alpha]^{25}_D$ +32.2 (*c* 0.02, $CHCl_3$); UV $\lambda_{max}$ (MeCN) (log $\epsilon$) 265 (4.05) nm; IR (neat) $\nu_{max}$ 3455 (br, OH), 1780, 1680 ($\alpha,\beta$-unsaturated $\gamma$-lactone), 1290 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 0.87 (3H, t, $J$ = 6.8 Hz, H-15′), 1.26 (22H, br s, H-4′−14′), 1.42−1.68 (4H, m, H-2′, 3′), 3.34 (3H, s, OMe-1′), 4.12 (1H, dd, $J$ = 7.4, 4.8 Hz, H-1′), 4.88, 5.20 (1H each, d, $J$ = 2.6 Hz, H-6a, b), 7.21 (1H, br s, H-4); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 14.3 (C-15′), 22.7 (C-14′), 25.5 (C-3′), 29.0−30.0 (C-4′−12′), 31.7 (C-13′), 35.2 (C-2′), 57.6 (OMe-1′), 77.3 (C-1′), 97.8 (C-6), 137.7 (C-4), 137.8 (C-3), 155.1 (C-5), 169.8 (C-2); EIMS *m*/*z* 336 $[M]^+$ (4), 280 (10), 267 (7), 179 (9), 165 (14), 149 (21), 142 (100), 123 (30), 111 (32), 97 (43), 83 (40), 69 (75), 55 (90); HREIMS *m*/*z* 336.2661 $[M]^+$ (calcd for $C_{21}H_{36}O_3$, 336.2664).

**Secosubamolide {methyl[(2*E*)-2-[(1*S*)-1-hydroxy-2-oxopropyl]-hexadec-2-enoate]} (4):** faint yellowish liquid; $[\alpha]^{25}_D$ +42.5 (*c* 0.15, $CHCl_3$); UV $\lambda_{max}$ (MeCN) (log $\epsilon$) 215 (3.80) nm; IR (neat) $\nu_{max}$ 3450 (br, OH), 1735 (ester), 1710 (ketone) $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 0.89 (3H, t, $J$ = 6.8 Hz, H-16), 1.25 (20H, br s, H-6−15), 1.63 (2H, m, H-5), 2.15 (3H, s, H-3′), 2.34 (2H, q, $J$ = 7.4 Hz, H-4), 3.73 (3H, s, OMe-1), 4.90 (1H, br s, H-1′), 7.08 (1H, t, $J$ = 7.6 Hz, H-3); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 14.1 (C-16), 22.6 (C-15), 24.4 (C-3′), 28.6 (C-4), 28.7 (C-5), 29.0−30.0 (C-6−13), 31.8 (C-14), 52.2 (OMe-1), 74.1 (C-1′), 129.5 (C-2), 149.4 (C-3), 166.5 (C-1), 206.3 (C-2′); FABMS *m*/*z* 341 $[M + H]^+$ (3), 309 (8), 297 (70), 265 (65), 247 (7), 237 (10), 219 (18), 191 (12), 167 (7), 155 (21), 149 (25), 125 (40), 115 (75), 97 (80), 83 (95), 69 (93), 55 (100); HRFABMS *m*/*z* 341.2688 $[M + H]^+$ (calcd for $C_{20}H_{37}O_4$, 341.2692).

**Cell Culture and Treatment.** The SW480 cell line was obtained from the American Type Culture Collection (Rockville, MD). The basal medium for the SW480 cell culture was DMEM supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin G, and 100 $\mu$g/mL streptomycin. Stock solutions of subamolide A (**1**), subamolide B (**2**), subamolide C (**3**), and secosubamolide (**4**) (100 mM) were dissolved in DMSO, and various concentrations were prepared in DMEM basal medium with a final DMSO concentration of 0.1%.

**DNA Damage and Cell Cycle Analysis on the SW480 Cell Line.** SW480 cells were cultured in 60 mm tissue-culture dishes. Culture medium was replenished when cells were 80% confluent. Monolayers were treated with various concentrations of subamolide A (**1**), subamolide B (**2**), subamolide C (**3**), and secosubamolide (**4**) for 24 h. Adherent and floating cells were collected after treatment, washed twice with PBS, then fixed with PBS−methanol (1:2, v/v) solution, and stored at 4 °C for at least 18 h. After two more washes with PBS, cell pellets were stained with PBS containing 50 $\mu$g/mL propidium iodide (PI) and 50 $\mu$g/mL DNase-free RNaseA for 30 min at room temperature in the dark. DNA fluorescence of PI-stained cells was evaluated by excitation at 488 nm and detected through a 630/22 nm band-pass filter using a Becton-Dickinson FACS-Calibur flow cytometer. At least 10 000 cells were examined per sample, and the DNA histograms were further analyzed using Modfit software on a Mac workstation to evaluate the extent of DNA damage and the percentage of cells in various phases ($G_0/G_1$, S, and $G_2$/M) of the cell cycle.

**Acknowledgment.** This investigation was supported by a grant from the National Science Council of the Republic of China, NSC-94-2320-B-242-009 (C.-Y.C.) and NSC-95-2320-B-415-005 (C.-H.C.).

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NP060425K