1873

Isolation and Characterization of Minor Analogues of Silvestrol and Other Constituents from a Large-Scale Re-collection of *Aglaia foveolata*

Li Pan,[†] Leonardus B. S. Kardono,[‡] Soedarsono Riswan,[§] Heebyung Chai,[†] Esperanza J. Carcache de Blanco,[†,⊥] Caroline M. Pannell,[||] Djaja Doel Soejarto,[▽] Thomas G. McCloud,[°] David J. Newman,[#] and A. Douglas Kinghorn*[·†]

Division of Medicinal Chemistry, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, United States, Research Center for Chemistry, Indonesian Institute of Science, Tangerang 15310, Indonesia, Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Science, Bogor 16122, Indonesia, Division of Pharmacy Practice and Administration, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, United States, Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RA, U.K., Program for Collaborative Research in the Pharmaceutical Science and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, United States, SAIC-Frederick, Inc., P.O. Box B, Frederick, Maryland 21702, United States, and National Cancer Institute, NCI-Frederick, Fairview Center, P.O. Box B, Frederick, Maryland 21702, United States

Two new minor silvestrol analogues [2‴-episilvestrol (**1**) and 2‴,5‴-diepisilvestrol (**2**)], together with a new 21-norbaccharane-type triterpene (**3**), two new 3,4-secodammarane triterpenes (**4** and **5**), and a new eudesmane sesquiterpene (**6**), as well as nine known compounds, were isolated from a large-scale re-collection of the CHCl₃-soluble extract of the stem bark of *Aglaia foveolata* obtained in Kalimantan, Indonesia. The structures of the new compounds were established by interpretation of their spectroscopic data. All of the isolates were tested for cytotoxicity against HT-29 cells. The new silvestrol analogues, **1** and **2**, were considerably less active as cytotoxic agents than silvestrol (**7**) and episilvestrol (5‴-episilvestrol) (**8**) against this cell line, showing the importance of the configuration at C-2‴ in mediating such activity within this compound class. Several of the compounds isolated were also evaluated in a NF-κB (p65) inhibition assay.

Silvestrol, a rocaglate derivative possessing a dioxanyloxy unit affixed to a cyclopenta[*b*]benzofuran skeleton, and its 5‴*S* epimer, episilvestrol, were isolated from the tropical tree *Aglaia foveolata* Pannell (Meliaceae) and fully characterized by Hwang et al. in 2004. The structure and absolute configuration of silvestrol were confirmed by single-crystal X-ray crystallography.[1] Silvestrol was found to possess comparable cytotoxic potencies for a small panel of human cancer cell lines to those of the well-known anticancer compounds paclitaxel (Taxol) and camptothecin and was further demonstrated as being active in the in vivo hollow fiber assay. This compound also showed activity in the P-388 lymphocytic leukemia test system in vivo, when administered both ip and iv.[1] In addition, silvestrol has been documented as an antineoplastic constituent of *Aglaia leptantha*, using human PC-3 prostate cancer cells in a murine xenograft experiment, but with only the planar structure of the dioxanyl ring moiety reported.[2]

In preliminary mechanistic studies, Swanson and associates demonstrated that silvestrol produces a p53-independent cell-cycle blockage at the G₂/M checkpoint using LNCaP human prostate cells.[3] In a follow-up investigation, silvestrol was shown to induce apoptosis in LNCaP cells via the involvement of caspases 2, 9, and 10, but not caspases 3 and 7.[4] More recently, silvestrol was found to exhibit B-cell selectivity in both chronic lymphocytic leukemia and acute lymphocytic leukemia models.[5] Silvestrol was observed to cause an early reduction in Mcl-1 expression in chronic lymphocytic leukemia cells from patients.[5] Additional mechanistic

investigation has indicated that silvestrol causes increased apoptosis, decreased proliferation, and inhibition of angiogenesis, and it inhibits the translation of malignancy-related mRNA by regulating the activity of initiation factor eIF4A.[6,7] The total synthesis of silvestrol has been accomplished independently by two different groups.[8−10]

The presence of the substituted 1,4-dioxanyloxy moiety in the silvestrol structure appears to be unique in nature and has been found essential for the exhibition of potent biological activity when compared with rocaglate derivatives lacking this functionality.[1,11] Thus far, only silvestrol and episilvestrol (5‴-epi-silvestrol) have been isolated with this structural feature among the cyclopenta[*b*]benzofuran derivatives from species in the genus *Aglaia*.[1,2,11]

Previous phytochemical work on the different plant parts of *A. foveolata*, collected in Kalimantan, Indonesia, has demonstrated that silvestrol occurs in the fruits, leaves, stem bark, and twigs of this species, with the highest yield (0.02% w/w) occurring in the stem bark.[1,12] In the present study, a large-scale re-collection of the stem bark of *A. foveolata* from Kalimanatan, Indonesia, was conducted to afford the scale-up isolation of silvestrol at the gram level, in order for more extensive biological testing to be performed. While this reisolation work was underway, an opportunity was taken to search for the presence of minor new analogues of silvestrol.

Cytotoxicity assay-guided fractionation of the CHCl₃-soluble extract of the stem bark of *A. foveolata* led to the isolation of two new minor silvestrol analogues (**1** and **2**), three new triterpenoids (**3−5**), and a new sesquiterpene (**6**), as well as nine known compounds. The structures of compounds **1−6** were established by spectroscopic data interpretation. Besides silvestrol and episilvestrol, the other known compounds were identified as 17,24-epoxy-25-hydroxybaccharan-3-one,[1] 17,24-epoxy-25-hydroxy-21-methoxy-3,4-secobaccharane,[12] eichlerianic acid,[13] cabraleone,[13] foveolin A,[13] methyl foveolate A (dymalol),[14] (−)-dehydrodiconiferyl alcohol,[15] and 3-oxo-15-hydroxy-T-muurolol,[16] by comparison of their spectroscopic data with published values. All of the isolates were tested for cytotoxicity against the human colon cancer cell

---

* To whom correspondence should be addressed. Tel: +1-614-247-8094. Fax: +1-614-247-8081. E-mail: kinghorn.4@osu.edu.
† Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University.
‡ Research Center for Chemistry, Indonesian Institute of Science.
§ Research Center for Biology, Indonesian Institute of Science.
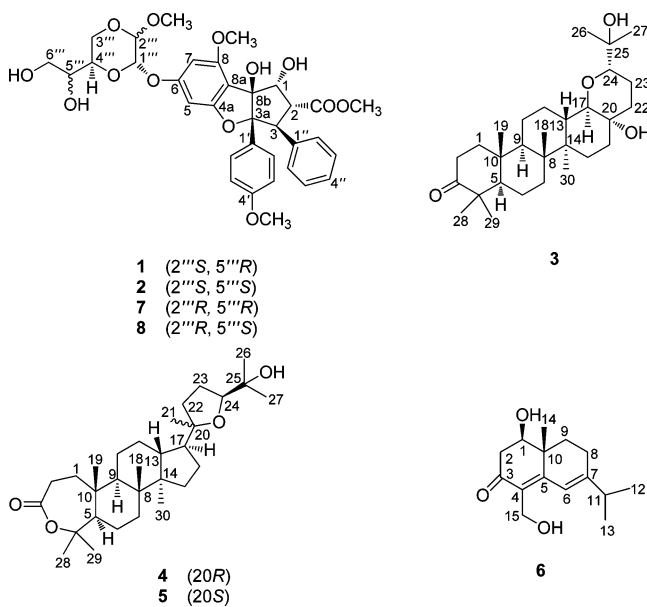⊥ Division of Pharmacy Practice and Administration, College of Pharmacy, The Ohio State University.
|| University of Oxford.
▽ University of Illinois at Chicago.
° SAIC-Frederick, Inc.
National Cancer Institute.

line (HT-29). The new compounds **3−6**, together with silvestrol and episilvestrol, were also evaluated in a NF-κB (p65) inhibition assay.

1	(2‴S, 5‴R)
2	(2‴S, 5‴S)
7	(2‴R, 5‴R)
8	(2‴R, 5‴S)

| 4 | (20R) |
| 5 | (20S) |

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds **1** and **2**[a]

	1		**2**	
position	δ_H, (J in Hz)	δ_C	δ_H, (J in Hz)	δ_C
1	5.04, d (7.2)	79.7	5.04, d (7.2)	79.7
2	3.90, dd (14.4, 6.6)	50.4	3.90, dd (14.4, 6.6)	50.2
3	4.27, d (14.4)	55.0	4.28, d (14.4)	55.0
3a		101.9		101.9
4a		160.4		160.6
5	6.48, d (1.8)	92.7	6.48, d (1.8)	92.9
6		160.0		160.0
7	6.40, d (1.8)	95.2	6.40, d (1.8)	95.4
8		157.1		157.1
8a		109.6		109.6
8b		93.3		93.4
1'		126.6		126.6
2',6'	7.09, d (9.0)	129.0	7.09, d (9.0)	129.0
3',5'	6.69, d (9.0)	112.8	6.69, d (9.0)	112.8
4'		158.8		158.8
1"		136.7		136.7
2",6"	6.84, m	127.8	6.85, m	127.8
3",5"	7.05, m	127.8	7.05, m	127.8
4"	7.05, m	126.6	7.06, m	126.6
1‴	5.40, brs	93.5	5.36, brs	92.7
2‴	4.65, d (1.2)	98.8	4.63, d (1.2)	98.9
3‴α	4.04, brd (12.0, 3.0)	66.5	4.24, dd (11.4, 1.8)	67.2
3‴β	4.01, t (11.0)		3.82, t (11.4)	
4‴	4.18, brd (10.2)	68.3	4.05, ddd (9.6, 7.8, 1.8)	67.4
5‴	3.70, brs	70.2	3.58, dd (10.8, 6.0)	71.2
6‴	3.66, d (10.8)	63.7	3.59−3.61, m	62.7
	3.68, d (10.8)		3.74, brd (10.8)	
COOCH$_3$-2		170.6		170.6
	3.65, s	52.1	3.65, s	52.1
OCH$_3$-8	3.87, s	56.0	3.87, s	56.0
OCH$_3$-4'	3.72, s	55.1	3.72, s	55.1
OCH$_3$-2‴	3.63, s	57.4	3.63, s	57.3

[a] ^{1}H NMR spectrum measured at 600 MHz, ^{13}C NMR spectrum measured at 150 MHz; obtained in CDCl$_3$ with TMS as internal standard. Assignments supported with HSQC and HMBC NMR spectra.

Results and Discussion

Compound **1** was obtained as a colorless gum with a negative specific rotation ([α]$^{20}_D$ −33.5, c 0.05, MeOH) and afforded a sodiated molecular ion peak at m/z 677.2233 [M + Na]$^+$ in the HRESIMS, corresponding to a molecular formula of C$_{34}$H$_{38}$O$_{13}$, the same as silvestrol. The ^{1}H and ^{13}C NMR spectra of compound **1** were observed to be very similar to those of silvestrol and episilvestrol.[1] In the ^{1}H NMR spectrum, 11 proton signals were detected in the low-field range from δ_H 6.30 to 7.20 ppm and were recognized as belonging to three aromatic rings, including two *meta*-coupled aromatic signals at δ_H 6.40 (1H, d, J = 1.8 Hz, H-7) and 6.48 (1H, d, J = 1.8 Hz, H-5), four AA′BB′-coupled proton signals of a 1,4-disubstituted phenyl group at δ_H 6.69 (2H, d, J = 9.0 Hz, H-3′ and H-5′) and 7.07 (2H, d, J = 9.0 Hz, H-2′ and H-6′), and proton signals of a monosubstituted phenyl ring at δ_H 6.84 (2H, m, H-2″ and H-6″) and 7.07 (3H, m, H-3″, 4″ and H-5″). The chemical shifts at δ_H 5.04 (1H, d, J = 7.2 Hz, H-1), 3.90 (1H, dd, J = 14.4, 6.6 Hz, H-2), and 4.27 (1H, d, J = 14.4 Hz, H-3) are typical signals of H-1, H-2, and H-3 of the five-membered carbocyclic ring of a cyclopenta[*b*]benzofuran unit (Table 1).[1] The above analysis of the ^{1}H NMR spectrum suggested the presence of a rocaglate unit in the molecule of **1**.[1] In the ^{13}C NMR spectrum of this compound, which was sorted using its DEPT and HSQC spectra, seven quaternary carbons at δ_C 160.4 (C-4a), 160.0 (C-6), 157.1 (C-8), 109.6 (C-8a), 126.6 (C-1′), 158.8 (C-4′), and 136.7 (C-1″) were found to be consistent with the occurrence of the three substituted benzene rings indicated above. The observed resonances of two oxygenated quaternary carbons at δ_C 101.9 (C-3a) and 93.4 (C-8b), two oxymethine groups at δ_C 79.7 (C-1) and 50.2 (C-2), and an alkyl methine group at δ_C 55.0 (C-3) are characteristic for a cyclopenta[*b*]benzofuran moiety.[1] Besides the rocaglate feature, the presence in **1** of an unusual [6-(1,2-dihydroxyethyl)-3-methoxy-1,4-dioxan-2-yl]oxy feature was also recognized, on the basis of the observation of oxygenated proton signals at δ_H 5.40 (1H, brs, H-1‴), 4.65 (1H, d, J = 1.2 Hz, H-2‴), 4.04 (1H, dd, J = 12.6, 3.0, H-3α‴), 4.01 (1H, t, J = 11.0, H-3β‴), 4.18 (1H, brd, J = 10.2 Hz, H-4‴), 3.70 (1H, brs, H-5‴), 3.66 (1H, d, J = 10.8 Hz, H$_a$-6‴), and 3.68 (1H, d, J = 10.8 Hz, H$_b$-6‴) in the ^{1}H NMR spectrum, as well as signals of four oxymethine carbons at δ_C 93.5 (C-1″), 98.8 (C-2‴), 68.3 (C-4‴), and 70.2 (C-5‴) and two oxymethylenes at δ_C 66.5 (C-3‴) and 63.7 (C-6‴) in the ^{13}C NMR

spectrum (Table 1). All of this information suggested that compound **1** is an isomer of silvestrol. By comparison of the ^{1}H NMR data of these two compounds, the major differences were evident in the 1,4-dioxanyloxy ring. A downfield shift of the methoxy group at C-2‴ from δ_H 3.48 to δ_H 3.63 was clearly discernible for **1**. Moreover, downfield shifts of 0.18 ppm for H-1‴, 0.09 ppm for H-2‴, and 0.51 ppm for H-3α‴, as well as an upfield shift of 0.10 ppm for H-3β‴, were also observed. For the rocaglate moiety of **1**, most proton signals were found to be almost identical to those of silvestrol, except for H-7 and H-5, two aromatic protons spatially close to the 1,4-dioxanyloxy ring, for which slight downfield shifts of 0.13 and 0.08 ppm, respectively, were observed. These subtle differences allowed the inference to be made that the methoxy group at C-2‴ on the 1,4-dioxanyloxy ring in **1** adopts an α-equatorial orientation rather than a β-axial orientation as in silvestrol. This presumption was consistent with the downfield shift of approximately 7.5 ppm for the carbon signal of C-3‴ (δ_C 66.5) in the ^{13}C NMR spectrum, due to the absence of the cis-γ substitution effect of the methoxy group on C-2‴ to H-3β‴. Furthermore, the α position of the methoxy group on C-2‴ was confirmed by the key NOE effects between H-2‴ and H-3β‴. Additional perusal of the ^{1}H NMR spectrum indicated that the proton signals of H-3α‴ (δ_H 4.04, 1H, dd, J = 12.0, 3.0 Hz), H-3β‴ (δ_H 4.01, 1H, t, J = 11.0 Hz), H-4‴ (δ_H 4.18, brd, J = 10.2 Hz), and H-5‴ (3.70, brs) adopted comparable splitting patterns and exhibited similar coupling constants to those for silvestrol (**7**), suggesting the configurations of C-4‴ and C-5‴ to be the same for both substances. This deduction was consistent with the analysis of the NOESY spectrum (Figure 1). Thus, the structure of compound **1** was determined to be 2‴-episilvestrol.

The HRESIMS of compound **2** showed a sodiated molecular ion peak at m/z 677.2212, corresponding to a molecular formula of C$_{34}$H$_{38}$O$_{13}$Na, the same as that of compound **1**. The NMR spectrum
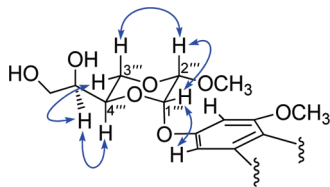
Figure 1. Selected NOESY ($\leftrightarrow$) correlations observed for **1**.

of compound **2** exhibited a very close resemblance to that of compound **1** and could be recognized as a second new silvestrol isomer (Table 1). The same α-orientation of the methoxy group on the 1,4-dioxane ring of **2** was deduced by the analysis of its 1D and 2D NMR spectroscopic data and by comparison with those of compound **1**. The major differences between compounds **1** and **2** were evident in the ethane 1,2-diol functionality on the 1,4-dioxanyloxy moiety. In the ^{1}H NMR spectrum of compound **2**, the proton signals of H-3α‴ (δ_H 4.24, 1H, dd, J = 11.4, 1.8 Hz) and H-3β‴ (δ_H 3.82, 1H, t, J = 11.4 Hz) adopted a similar coupling pattern to that in compound **1** and silvestrol, while the splitting patterns of H-4‴ (δ_H 4.05, ddd, J = 9.6, 7.8, 1.8) and H-5‴ (δ_H 3.58, 1H, dd, J = 10.8, 6.0) were comparable with those of the known 5‴-epimer of silvestrol (episilvestrol).[1] Thus, compound **2** was determined to be 2‴,5‴-diepisilvestrol. This structural proposal was confirmed by analysis of its HSQC, HMBC, and NOESY 2D-NMR spectra.

Compound **3** was obtained as a white, amorphous powder with a molecular formula of $C_{29}H_{48}O_4$, as determined from the sodiated molecular ion peak at m/z 483.3431 [M + Na]$^+$ in the HRESIMS. The ^{1}H NMR spectrum of **3** exhibited signals for seven tertiary methyl groups at δ_H 0.96 (3H, s, H-19), 0.98 (3H, s, H-30), 1.03 (3H, s, H-29), 1.07 (3H, s, H-18), 1.08 (3H, s, H-28), 1.16 (3H, s, H-26), and 1.20 (3H, s, H-27), two typical methine protons at δ_H 3.26 (1H, d, J = 12.0 Hz) and 3.48 (1H, d, J = 11.4 Hz), and a group of highly overlapped alkyl protons in the high field that occurred in the range from δ_H 1.20 to 2.60 (Table 2). Altogether, 29 carbon signals in the ^{13}C NMR spectrum were sorted by DEPT and HSQC into seven methyls, 10 methylenes, three methines, four quaternary carbons, two oxygenated methines (δ_C 73.8, C-17 and 81.9, C-24), two quaternary oxygenated carbons (δ_C 69.2, C-20 and 71.9, C-25), and a ketone group (δ_C 217.7, C-3) (Table 3). These characteristic signals as observed in the ^{1}H NMR and ^{13}C NMR spectra were comparable with those of 17,24-epoxy-25-hydroxybaccharan-3-one, which is a baccharane-type triterpene first isolated from *A. foveolata* in an earlier study, and structurally confirmed by X-ray crystallographic analysis.[1] Comparison of the ^{1}H NMR and ^{13}C NMR spectroscopic data of **3** with those of this known compound revealed that the only difference was the absence of a tertiary methyl group (C-21) at C-20 and the presence of an oxygenated quaternary carbon (δ_C 69.2) instead of an alkyl quaternary carbon. The above observations, together with the molecular formula discerned from the HRESIMS, suggested that compound **3** is a baccharane-type nor-triterpene, with the C-21 methyl group having been replaced by a hydroxy group. In order to obtain further information on the free hydroxy groups attached at the quaternary carbons, CDCl$_3$ and DMSO-d_6 were both used as NMR solvent. In the ^{1}H NMR spectrum with DMSO-d_6 as solvent, the hydroxy proton signals at C-20 and C-24 appeared at δ_H 4.17 and 3.97, respectively. HMBC correlations were observed between the signal for OH-20 and C-20, C-17, C-16, and C-22 and between OH-24 and C-25, C-26, and C-27 and confirmed the locations of these two hydroxy groups (Figure 2). In the NOESY spectrum, enhancements of OH-20 with H-17, H-22α, and H-23α suggested an α-orientation of OH-20. In addition, NOE effects were also observed between CH$_3$-19 and CH$_3$-18, CH$_3$-18 and H-13, H-17 and CH$_3$-30, and H-24 and H-22β, which were consistent with the relative configuration of the known baccharane-type triterpenes (Figure 2).[1] In the CD spectrum of **3**, a positive n−π* Cotton effect

Table 2. ^{1}H NMR Chemical Shifts of Compounds **3−6**^{a}

position	3	4	5	6
1	1.48^b	1.62^b	1.63^b	3.88, m
	1.95, m	1.82^b	1.82^b	
2	2.46^b	2.65, td	2.67, td	H$_\alpha$, 2.72,
		(14.0, 4.6)	(14.0, 4.5)	dd (6.1, 17.3)
	2.50, ddd	2.52, ddd	H$_\beta$, 2.66,
		(14.2, 5.7, 3.5)	(14.2, 5.7, 3.5)	dd (12.1, 17.3)
5	1.35^b	1.78^b	1.80^b	
6	1.50^b	1.43^b	1.44^b	6.46, s
		1.77^b	1.77^b	
7	1.42^b	1.30^b	1.31^b	
8				2.30, m
9	1.43^b	1.42^b	1.42^b	H$_\alpha$, 1.43,
				ddd (6.0, 5.7, 12.5)
				H$_\beta$, 2.16,
				ddd (2.0, 5.0, 12.8)
11	1.35^b	1.55^b	1.55^b	2.46, hep (6.6)
	1.55^b	1.26^b	1.26^b	
12		1.19^b	1.10^b	1.12, d (6.8)
		2.03,	1.86^b	1.12, d (6.8)
		brd (10.0)		
13	2.07, td	1.75^b	1.70^b	
	(12.0, 3.0)			
14				1.09, s
15	1.58^b	1.10^b	1.10^b	4.45, brs
	1.20^b	1.45^b	1.45^b	
16	1.60^b	1.30^b	1.30^b	
	1.75^b	1.78^b	1.78^b	
17	3.48, d (11.4)	1.79^b	1.91^b	
18	1.07, s	1.02, s	1.05, s	
19	0.96, s	1.08, s	1.10, s	
21		1.15, s	1.17, s	
22	H$_\alpha$1.48^b	1.71^b	1.71^b	
	H$_\beta$ 2.03, td			
	(13.2, 4.8)			
23	H$_\beta$1.56^b	1.80^b	1.88^b	
	H$_\alpha$1.73^b		1.82^b	
24	3.26, d (12.0)	3.77,	3.65, dd	
		t (7.2)	(9.9, 5.6)	
26	1.16, s	1.20, s	1.21, s	
27	1.20, s	1.10, s	1.13, s	
28	1.08, s	1.48, s	1.50, s	
29	1.03, s	1.40, s	1.42, s	
30	0.98, s	0.86, s	0.88, s	

^{a} Measured at 600 MHz and obtained in CDCl$_3$ with TMS as internal standard; J values (Hz) are given in parentheses. Assignments supported with ^{1}H−^{1}H COSY, HSQC, and HMBC spectra. ^{b} Multiplicity patterns unclear due to signal overlapping.

was found around 289 nm, suggesting that this compound adopts the same absolute configuration as found in structurally closely related 3-oxotriterpenoids.[17,18] Accordingly, the structure of compound **3** was elucidated as 17,24-epoxy-20α,25-dihydroxy-21-norbaccharan-3-one.

Compound **4** was obtained as a white, amorphous resin. The molecular formula was determined to be $C_{30}H_{50}O_4$, on the basis of the sodiated molecular ion peak at m/z 497.3651 [M + Na]$^+$ in the HRESIMS. Its ^{1}H NMR spectrum exhibited signals for eight tertiary methyl groups at δ_H 0.86 (3H, s, H-30), 1.02 (3H, s, H-18), 1.08 (3H, s, H-19), 1.10 (3H, s, H-27), 1.15 (3H, s, H-21), 1.20 (3H, s, H-26), 1.40 (3H, s, H-29), and 1.48 (3H, s, H-28), an oxygen-bearing methine group at δ_H 3.77 (1H, t, J = 6.9 Hz, H-24), a methylene vicinal to a carbonyl group at δ_H 2.65 (1H, td, J = 14.0, 4.6, H-2α) and 2.50 (1H, ddd, J = 14.2, 5.7, 3.5, H-2β), and a number of alkyl protons in the high-field region at δ_H 1.0−2.2 ppm (Table 2). The 30 carbon signals in the ^{13}C NMR spectrum were classified by DEPT and HSQC NMR experiments into eight methyls, 10 methylenes, four methines, three quaternary carbons, four-oxygen bearing carbons (including one secondary and three tertiary), and a carbonyl group. The NMR data of compound **4** were characteristic of the resonances of a 3,4-secodammarane derivative with a tetrahydrofuran (20,24-epoxy) ring formed by the closure of a side chain attached to the D ring.[11,17] Compounds based on this triterpenoid skeleton have been obtained previously from certain species belonging to the genera *Aglaia* and *Cabralea* of the plant family Meliaceae.[11,17] On comparing the NMR data of **4** with those of the structurally closely related known compound foveolin B, a

Table 3. ^{13}C NMR Chemical Shifts of Compounds **3−6**^{a}

position	3	4	5	6
1	39.7	40.7	40.3	71.8
2	34.0	32.8	32.4	42.4
3	217.7	175.5	175.0	198.7
4	47.3	86.4	86.0	129.4
5	54.9	53.5	53.1	157.9
6	19.6	23.9	23.6	116.9
7	33.0	34.6	34.2	160.3
8	42.4	40.5	40.2	23.7
9	50.0	51.7	51.4	32.0
10	36.9	39.7	39.3	38.7
11	21.2	23.1	23.0	36.4
12	23.7	27.3	27.2	21.4
13	35.2	43.5	43.1	20.8
14	40.8	50.2	49.9	56.2
15	27.5	31.4	30.9	15.0
16	33.8	26.1	25.7	
17	81.9	50.3	49.6	
18	15.6	15.3	14.9	
19	16.2	18.8	18.4	
20	69.2	86.8	86.5	
21		22.1	27.2	
22	29.1	37.8	34.8	
23	20.1	26.2	26.3	
24	73.8	84.9	86.4	
25	71.9	71.5	70.2	
26	24.0	27.6	27.8	
27	26.3	24.4	24.0	
28	26.8	31.3	30.9	
29	21.0	26.7	26.9	
30	14.4	16.2	16.1	

^{a} Measured at 150 MHz and obtained in CDCl$_3$ with TMS as internal standard. Assignments supported with HSQC and HMBC NMR spectra.
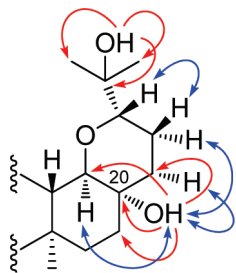
Figure 2. Selected HMBC (→) and NOESY (↔) correlations observed for **3**.

known constituent of *A. foveolata*, it was revealed that the major differences were evident in the A ring.[11] In the ^{13}C NMR spectrum, the signals of the methylene groups at C-1 and C-2 were shifted downfield by 5.8 and 5.0 ppm, respectively, while the C-3 carbonyl group resonance was shifted upfield by 4.2 ppm. In turn, the signal of the quaternary oxygenated C-4 carbon was also shifted downfield by nearly 10 ppm. These differences, together with the molecular formula of compound **4**, suggested the possibility of an ester bond between the carbonyl group of C-3 and the quaternary oxygenated carbon of C-4, resulting in the closure of the 3,4-seco ring to form a seven-membered lactone. In the HMBC spectrum, correlations were observed from H-1, H-2, CH$_3$-28, and CH$_3$-29 to the carbonyl group (δ_C 175.5, C-3), from CH$_3$-28 and CH$_3$-29 to C-4 and C-5, and from CH$_3$-19 to C-1, C-10, and C-9 and provided confirmatory evidence for this proposal (Figure 3). According to the previous literature, ^{13}C NMR chemical shifts of the carbons of the epoxide ring, as well as the coupling pattern of H-24, have been used to determine the absolute configuration of the 20,14-epoxy group of 3,4-secodammarane derivatives.[13] In the ^{13}C NMR spectrum of **4**, the signals of C-20, C-21, C-22, C-23, and C-24 appeared at δ_C 86.8, 22.1, 37.8, 26.2, and 84.9, respectively (Table 3). In turn, in the ^{1}H NMR spectrum, the H-24 signal was exhibited as a triplet with a *J* value of 6.8 Hz. This information was supportive of a 20*R*, 24*S* configuration of the epoxy group, as described previously.[13] These assignments were supported by the key NOESY
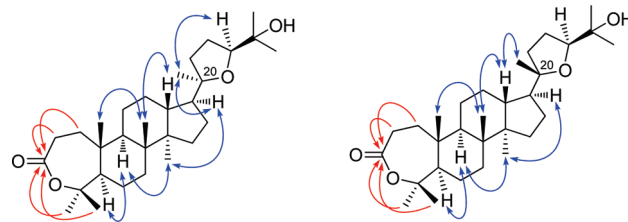
Figure 3. Selected HMBC (→) and NOESY (↔) correlations observed for **4** and **5**.

correlations between CH$_3$-21 and both H-17 and H-24, respectively. Additional NOE cross-peaks of H-5 and H-9, H-9 and CH$_3$-30, CH$_3$-18 and CH$_3$-19, H-13 and CH$_3$-19, and H-17 and CH$_3$-30 were used to establish the relative configuration of the remaining stereocenters of compound **4** (Figure 3), which were identical to those of known derivatives.[13,19] Thus, the structure of compound **4** was determined to be 20*R*,24*S*-epoxy-25-hydroxy-A-homo-4-oxadammaran-3-one.

The molecular formula of compound **5** was determined to be C$_{30}$H$_{50}$O$_4$, the same as that of compound **4**, from the sodiated molecular ion peak at *m/z* 497.3639 in the HRESIMS. The ^{1}H and ^{13}C NMR spectra of **5** were almost identical to those of **4** except for some slight but distinctive differences in the epoxide ring signals, which suggested that compound **5** is a stereoisomer of **4** (Tables 2 and 3). The ^{13}C NMR resonances of C-20, C-21, C-22, C-23, C-24, and C-25 were assigned with chemical shift values of δ_C 86.5, 27.2, 34.8, 26.3, and 86.4, respectively, and the proton signal for H-24 appeared as a double doublet with coupling constants of 9.9 and 5.6 Hz (Tables 2 and 3). The typical NMR parameters of the epoxy ring corresponded to the 20*S*, 24*S* configurations, as described in the literature.[13] In the NOESY spectrum, correlations between CH$_3$-21 and H-24 as well as H-17 as in compound **4** were absent, while an enhancement between CH$_3$-21 and H-13 was observed, consistent with a configurational change (Figure 3). Other important NOE effects observed for **5** were similar to those observed for **4**. Consequently, compound **5** was designated as 20*S*,24*S*-epoxy-25-hydroxy-A-homo-4-oxadammaran-3-one, the 20*S* epimer of compound **4**.

Compound **6** was obtained as a colorless resin. The HRESIMS of **6** afforded a sodiated molecular ion peak at *m/z* 273.1441, corresponding to an elemental formula of C$_{15}$H$_{22}$O$_3$Na. In the ^{1}H NMR spectrum, the resonance at δ_H 6.46 (1H, s, H-6) was ascribed as a signal from an endocyclic double bond, and the proton signals at δ_H 3.88 (1H, m, H-2) and 4.45 (2H, brs, H-15) suggested the presence of an oxymethine and an oxymethylene, respectively. Besides a tertiary methyl group signal at δ_H 1.09 (3H, s, H-14), an isopropyl group could be recognized on the basis of the proton signals of two secondary methyl groups at δ_H 1.09 (2 × 3H, d, *J* = 6.8 Hz) and a heptet at δ_H 1.09 (1H, *J* = 6.6 Hz). The 15 carbon signals observed in the ^{13}C NMR spectrum were sorted by DEPT and HSQC into three methyls, three methylenes, a methine, a quaternary carbon, two oxygenated carbons (including one primary and one secondary), a conjugated ketone group, a trisubstituted double bond, and a tetrasubstituted double bond. These NMR spectroscopic observations suggested that compound **6** is a sesquiterpenoid. In its ^{1}H−^{1}H COSY spectrum, the H-1 signal coupled with the geminal protons of H-2α (δ_H 2.72, 1H, dd, *J* = 6.1, 17.3) and H-2β (δ_H 2.16, 1H, *J* = 12.1, 17.3), which were located next to the ketone group (δ_C 198.7, C-3). The geminal protons of H-9α (δ_H 1.43, 1H, ddd, *J* = 5.7, 6.0, 12.5) and H-9β (δ_H 2.16, 1H, *J* = 2.0, 5.0, 12.5) showed coupling to the allylic methylene protons at δ_H 2.30 (2H, m, H-8). The partial structures were further connected by HMBC correlations from CH$_3$-14 to C-5, C-2, and C-9, from H-13 and H-12 to C-7, from H-6 to C-4 and C-5, and from H-15 to C-5, C-4, and C-3 (Figure 4). Compound **6** was therefore elucidated as a 4,6-diene eudesmane derivative, with C-3 substituted
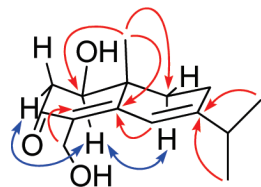
Figure 4. Selected HMBC (→) and NOESY (↔) correlations observed for **6**.

Table 4. Cytotoxicity of Compounds Isolated from the Stem Bark of *Aglaia foveolata*[a]

compound	HT-29[b]
5‴-episilvestrol (**1**)	2.29
2‴,5‴-diepisilvestrol (**2**)	1.07
silvestrol (**7**)	0.0007
2‴-episilvestrol (**8**)	0.001
paclitaxel[c]	0.0006
camptothecin[c]	0.06

[a] Compounds **3**−**6** and all other known compounds obtained in this investigation were inactive against HT-29 cells (ED$_{50}$ >10 μM). [b] Results are expressed as ED$_{50}$ values (μM). [c] Used as a positive control substance.

by a ketone group, and hydroxy groups present at C-1 and C-15, respectively. The β-orientation of the hydroxy group located at C-1 was deduced by the observed NOE effects of H-1 with H-2α and H-9α (Figure 4). A positive Cotton effect was observed in the range 250 to 375 nm in the CD spectrum of **6**, consistent with that of β-cyperone, a known 4,6-dien-3-one eudesmane.[20] Accordingly, the absolute configuration of **6** was established as 1*R*, 10*R*, as shown. Therefore, the structure of compound **6** was determined to be 4,6-diene-1,15-dihydroxyeudesma-3-one.

All pure compounds obtained in the present investigation were evaluated for their cytotoxic activity against the HT-29 human colon cancer cell line (Table 4). Among these compounds, the highly active agents silvestrol (**7**) and 5‴-episilvestrol (**8**) exhibited potent cytotoxicity, with ED$_{50}$ values 0.0007 and 0.001 μM, respectively. The minor new compounds **1** and **2**, the C-2‴ epimers of silvestrol and 5‴-episilvestrol, were found to be much less active, with ED$_{50}$ values of 2.3 and 1.1 μM, respectively. Accordingly, it is noted that when the axial methoxy group on the 1,4-dioxanyloxy ring at C-2‴ in silvestrol and 5‴-episilvestrol was changed to an equatorial orientation, as in compounds **1** and **2**, the resultant cytotoxicity decreased dramatically. This investigation has thus demonstrated that the configuration of the chiral carbon C-2‴ in the 1,4-dioxanyloxy unit of silvestrol derivatives plays an important role in mediating biological activity among these plant secondary metabolites. An enzyme-based ELISA NF-κB assay was also employed to test the p65 (RelA) inhibitory activity of the new compounds **3**−**6**, in addition to silvestrol and 5‴-episilvestrol. All of these substances exhibited IC$_{50}$ values of >20 μM and were considered inactive. Compounds **1** and **2** were not tested in the NF-κB assay due to the very small amounts isolated.

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a Perkin-Elmer 343 automatic polarimeter. UV spectra were recorded with a Perkin-Elmer Lambda 10 UV/vis spectrometer. CD spectra were run on a JASCO J-810 spectrometer. NMR spectroscopic data were obtained on a Bruker Avance DRX-400 or -600 MHz spectrometer. IR spectra were measured on a Thermo Scientific Nicolet 6700 FT-IR spectrometer. Column chromatography was performed with 65−250 or 230−400 mesh silica gel (Sorbent Technologies, Atlanta, GA). Analytical thin-layer chromatography was conducted on precoated, 250 μm thick silica gel plates (UV$_{254}$, glass backed, Sorbent Technologies, Atlanta, GA). Analytical HPLC was conducted on a 150 mm × 4.6 mm i.d. Sunfire PrepC$_{18}$ column (Waters, Milford, MA), and semipreparative HPLC was conducted on a 150 mm × 19 mm i.d., 5 μm

Sunfire PrepC$_{18}$ column (Waters, Milford, MA), along with a Waters system equipped with a 600 controller, a 717 Plus autosampler, and a 2487 dual wavelength absorbance detector.

Plant Material. A re-collection of *A. foveolata* stem bark was done in the autumn of 2007, in Kalimantan, Indonesia, by S.R. (Herbarium Bogoriense, Bogor, Indonesia), through the cooperation of L.B.S.K (LIPI, Tangerang, Indonesia). A voucher specimen (AA6126) has been deposited at the Herbarium of the Field Museum of Natural History, Chicago, IL.

Extraction and Isolation. The dried stem bark of *A. foveolata* (40−45 kg) was ground at the University of Illinois Pharmacognosy Field Station, Downers Grove, IL, where it was also bulk extracted with MeOH. The MeOH extract was reduced in volume, and 20% (4 L) was dispatched for isolation work at The Ohio State University. This MeOH extract (4 L) of stem bark of *A. foveolata* was concentrated under reduced pressure to yield 2 kg of thick, dark brown syrup. A part of the extract (500 g) was partitioned sequentially with hexane (3 × 1 L) and CHCl$_3$ (3 × 1 L). The CHCl$_3$ partition was washed with 1% saline solution to yield 200 g of a partially detannified CHCl$_3$-soluble extract, which was found to be active against the HT-29 cell line (ED$_{50}$ 0.4 μg/mL). Accordingly, part of this fraction (180 g) was subjected to separation over a silica gel column (11 × 100 cm, CH$_2$Cl$_2$−acetone, 20:1 to 100% acetone) to yield eight fractions (F01−F08). Fraction F07 (HT-29 cell line, ED$_{50}$ < 0.16 μg/mL, 7 g) was chromatographed over a LH-20 gel column (5 × 50 cm; eluted with 100% MeOH), to furnish three pooled subfractions (F701−F703). Subfraction F703 was demonstrated by TLC to be a silvestrol-rich subfraction and was subjected to separation over a preparative RP-18 column (150 mm × 19 mm i.d.), using MeOH−H$_2$O (55:45, 8 mL/min) as solvent, to afford compounds **1** (0.8 mg; t_R = 20.5 min), **2** (0.9 mg; t_R = 26.0 min), silvestrol (**7**, 100 mg; t_R = 36.5 min), and episilvestrol (**8**, 4.0 mg; t_R = 42.5 min). Subfraction F702 was chromatographed on an open ODS column (2 × 15 cm) with a MeOH−H$_2$O gradient solvent system (50:50 to 90:10), to yield compounds **3** (5.0 mg) and 17,24-epoxy-25-hydroxybaccharan-3-one (4.0 mg).

The bulk of the MeOH extract prepared at the University of Illinois at Chigaco (ca. 80%) was transferred to SAIC-Frederick, Inc. Thus, an 11 kg MeOH extract of *A. foveolata* was subjected to separation on a silica gel column to yield 154 fractions, from which nine silvestrol-enriched subfractions were retained for the purification of gram quantities of silvestrol. Follow-up isolation work on the remaining 145 side-cut fractions was conducted at The Ohio State University. The active fraction F112 (HT-29 cell line, ED$_{50}$ 1.1 μg/mL, 110 g) was chromatographed on a silica gel column, using CH$_2$Cl$_2$−acetone mixtures for elution, to yield eight subfractions (F11201−F11208). The presence of silvestrol and episilvestrol was detected by HPLC analysis in subfractions F11206 and F11207, respectively [C$_{18}$ column, 150 mm × 4.6 mm i.d.; MeOH−H$_2$O 60:40; flow rate 1.5 mL/min; t_R (silvestrol) = 6.0 min; t_R (episilvestrol) = 6.7 min]. Subfraction F11203 was chromatographed on a silica gel column, using CHCl$_3$−acetone mixtures for elution, to give 17,24-epoxy-25-hydroxy-21-methoxy-3,4-secobaccharane (50 mg), eichlerianic acid (20 mg), and cabraleone (3.5 mg).

In an attempt to find additional new compounds, two side-cut subfractions, F115-38-21 and F115-38-26 (1.0 g), were also investigated. These were prepared at SAIC-Frederick, Inc., from a silvestrol-containing fraction, F115, by passage over a RP-8 column using a CH$_3$CN−H$_2$O gradient eluent. Subfraction F115-38-21 (500 mg) was chromatographed on a semipreparative RP-18 column by HPLC, using MeOH−H$_2$O (50:50, 5 mL/min) as solvent system, to give (−)-dehydrodiconiferyl alcohol (3.5 mg, t_R = 12.5 min), **6** (1.2 mg, t_R = 15.5 min), and 3-oxo-15-hydroxy-T-muurolol (1.0 mg, t_R = 19.4 min). Subfraction F115-38-26 (1.0 g) was subjected repeatedly to silica gel columns, using CH$_2$Cl$_2$−acetone gradient solvent systems (20:1 to 1:1) for elution, to afford compounds **4** (4.0 mg), **5** (3.8 mg), foveolin A (15 mg), and methyl foveolate A (dymalol, 6 mg).

2‴-Episilvestrol (1): colorless gum; [α]$^{20}_D$ −33.5 (*c* 0.05, MeOH); UV (MeOH) λ$_{max}$ (log ε) 211 (4.54), 233 (4.07), 278 (3.19) nm; CD (*c* 3.06 × 10^{-5} M, MeOH) λ$_{max}$ (Δε) 217 (−6.05), 250 (+1.86), 294 (+2.40) nm; IR (film) ν$_{max}$ 3460, 2919, 2850, 1733, 1717, 1683, 1616, 1558, 1540, 1507, 1457, 1251, 1217, 1119, 1057, 1031, 753 cm^{-1}; ^{1}H NMR (600 MHz, CDCl$_3$) and ^{13}C NMR (150 MHz, CDCl$_3$) data, see Table 1; HRESIMS *m/z* 677.2233 [M + H]$^+$ (calcd for C$_{34}$H$_{38}$O$_{13}$Na, 677.2210).

2‴,5‴-Diepisilvestrol (2): colorless gum; $[\alpha]^{20}_D$ −53.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.51), 232 (4.04), 278 (3.23) nm; CD (*c* 3.06 × 10^{-5} M, MeOH) λ_{max} (Δε) 215 (−7.85), 251 (+1.20), 292 (+1.72) nm; IR (film) ν_{max} 3461, 2926, 2850, 1734, 1717, 1683, 1653, 1616, 1558, 1540, 1507, 1457, 1250, 1118, 1043, 753 cm^{-1}; ^{1}H NMR (600 MHz, CDCl$_3$) and ^{13}C NMR (150 MHz, CDCl$_3$) data, see Table 1; HRESIMS *m/z* 677.2212 [M + H]$^+$ (calcd for C$_{34}$H$_{38}$O$_{13}$Na, 677.2210).

17,24-Epoxy-20α,25-dihydroxy-21-norbaccharan-3-one (3): colorless gum; $[\alpha]^{20}_D$ +77.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.10) nm; CD (*c* 1.09 × 10^{-4} M, MeOH) λ_{max} (Δε) 289 (+1.31) nm; IR (film) ν_{max} 3395, 2948, 2868, 1701, 1456, 1384, 1161, 1067, 991, 946, 753 cm^{-1}; ^{1}H NMR (600 MHz, CDCl$_3$) and ^{13}C NMR (150 MHz, CDCl$_3$) data, see Tables 2 and 3; HRESIMS *m/z* 483.3431 [M + Na]$^+$ (calcd for C$_{29}$H$_{48}$O$_4$Na, 483.3450).

20S,24S-Epoxy-25-hydroxy-A-homo-4-oxadammaran-3-one (4): colorless gum; $[\alpha]^{20}_D$ +77.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.19) nm; CD (*c* 2.09 × 10^{-3} M, MeOH) λ_{max} (Δε) 210 (+0.52), 283 (+0.03) nm; IR (film) ν_{max} 3420, 2939, 2870, 1717, 1457, 1387, 1374, 1286, 1139, 1111, 1027, 755 cm^{-1}; ^{1}H NMR (600 MHz, CDCl$_3$) and ^{13}C NMR (150 MHz, CDCl$_3$) data, see Tables 2 and 3; HRESIMS *m/z* 497.3651 [M + Na]$^+$ (calcd for C$_{30}$H$_{50}$O$_4$Na, 497.3607).

20S,24S-Epoxy-25-hydroxy-A-homo-4-oxadammaran-3-one (5): colorless gum; $[\alpha]^{20}_D$ +111.0 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.16) nm; CD (*c* 2.09 × 10^{-4} M, MeOH) λ_{max} (Δε) 214 (+0.28), 288 (+0.12) nm; IR (film) ν_{max} 3423, 2967, 2933, 2865, 1718, 1457, 1387, 1374, 1288, 1143, 1111, 1058, 755 cm^{-1}; ^{1}H NMR (600 MHz, CDCl$_3$) and ^{13}C NMR (150 MHz, CDCl$_3$) data, see Tables 2 and 3; HRESIMS *m/z* 497.3639 [M + Na]$^+$ (calcd for C$_{30}$H$_{50}$O$_4$Na, 497.3607).

4,6-Diene-1β,15-dihydroxyeudesma-3-one (6): colorless gum; $[\alpha]^{20}_D$ +217.0 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.65), 302 (4.42) nm; CD (*c* 1.00 × 10^{-4} M, MeOH) λ_{max} (Δε) 295 (+4.3) nm; IR (film) ν_{max} 3406, 2962, 2917, 2873, 1645, 1613, 1569, 1465, 1423, 1362, 1328, 1295, 1220, 1035, 997, 755 cm^{-1}; ^{1}H NMR (600 MHz, CDCl$_3$) and ^{13}C NMR (150 MHz, CDCl$_3$) data, see Tables 2 and 3; HRESIMS *m/z* 273.1441 [M + Na]$^+$ (calcd for C$_{15}$H$_{22}$O$_3$Na, 273.1467).

Cell Culture and Cytotoxicity Assay. Human colon cancer cells (HT-29) were obtained from American Type Collection (ATCC catalog no. HTB-38). Cells were cultured in MEME medium (Hyclone, Logan, UT) supplemented with streptomycin (100 μg/mL), penicillin (100 units/mL), amphotericin B (Fungizone, 0.25 μg/mL), and 10% fetal bovine serum (FBS) and incubated in a humidified incubator with an atmosphere of 95% air and 5% CO$_2$ at 37 °C. Cells were trypsinized and split for subculture when they reached near-confluent state (five days or later). Upon reaching about 60−70% confluence, the medium was changed and the cells were used for test procedures one day later.

The harvested cells, after appropriate dilutions, were seeded in 96-well (9500 cells/190 μL) plates using complete medium and treated with the test compounds (10 μL/well in triplicate) at various concentrations. Test samples were initially dissolved in DMSO and then diluted 10-fold with H$_2$O. Serial dilutions were performed using 10% DMSO as the solvent. For the control groups, 10 μL of 10% DMSO was also added to each well. The plates were incubated for three days at 37 °C in 5% CO$_2$. On the third day, the cells were fixed to the plates by the addition of 100 μL of cold 20% trichloroacetic acid and incubated at 4 °C for 30 min. The plates were washed three times with tap water and dried overnight. The fixed cells were dyed with sulforhodamine B (SRB, an anionic protein stain) solution at 0.4% (w/v) in 1% acetic acid and incubated at room temperature for 30 min. The plates were washed three times with 1% acetic acid and allowed to air-dry. The bound SRB stain was then solubilized with 10 mM unbuffered Tris base (pH 10, 200 μL/well). The plates were placed on a shaker for 5 min, and the absorbance was read at 515 nm using a Bio-Tek μQuant microplate reader. The ED$_{50}$ values of test samples with serial dilutions were calculated using nonliner regression analysis (Table Curve2Dv4; AISN Software, Inc., Mapleton, OR).

Enzyme-Based ELISA NF-κB Assay. The NF-κB p65 subunit inhibitory activity of five major compounds (3−8) was tested in an enzyme-based ELISA NF-κB assay, which was carried out according to a published protocol.[22,23] Rocaglamide was used as a positive control, with an ED$_{50}$ value of 0.08 μM in this assay.

Supporting Information Available: Comparison of ^{1}H NMR spectra of four silvestrol derivatives (**1**, **2**, **7**, and **8**); ^{1}H and ^{13}C NMR and selected 2D NMR spectra of new compounds **1−6**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Hwang, B. Y.; Su, B. N.; Chai, H.-B.; Mi, Q.; Kardono, L. B. S.; Afriastini, J. J.; Riswan, S.; Santarsiero, B. D.; Mesecar, A. D.; Wild, R.; Fairchild, C. R.; Vite, G. D.; Rose, W. C.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Swanson, S. M.; Kinghorn, A. D. *J. Org. Chem.* **2004**, *69*, 3350−3358; 6156.

(2) Meurer-Grimes, B. M.; Yu, J.; Vairo, G. L. U.S patent 6710075 B2, 2004.

(3) Mi, Q.; Kim, S.; Hwang, B. Y.; Su, B.-N.; Chai, H.-B.; Arbieva, Z. H.; Kinghorn, A. D.; Swanson, S. M. *Anticancer Res.* **2006**, *26*, 3349−3356.

(4) Kim, S.; Hwang, B. Y.; Su, B.-N.; Chai, H.-B.; Mi, Q.; Kinghorn, A. D.; Wild, R.; Swanson, S. M. *Anticancer Res.* **2007**, *27*, 2175−2183.

(5) Lucas, D. M.; Edwards, R. B.; Lozanski, G.; West, D. A.; Shin, J. D.; Vargo, M. A.; Davis, M. E.; Rozewski, D. M.; Johnson, A. J.; Su, B. N.; Goettl, V. M.; Heerema, N. A.; Lin, T. S.; Lehman, A.; Zhang, X. L.; Jarjoura, D.; Newman, D. J.; Byrd, J. C.; Kinghorn, A. D.; Grever, M. R. *Blood* **2009**, *113*, 4656−4666.

(6) Bordeleau, M. E.; Robert, F.; Gerard, B.; Lindqvist, L.; Chen, S. M. H.; Wendel, H. G.; Brem, B.; Greger, H.; Lowe, S. W.; Porco, J. A., Jr.; Pelletier, J. *J. Clin. Invest.* **2008**, *118*, 1−11.

(7) Cencic, R.; Carrier, M.; Galicia-Vázquez, G.; Bordeleau, M.-E.; Sukarieh, R.; Bourdeau, A.; Brem, B.; Teodoro, J. G.; Greger, H.; Tremblay, M. L.; Porco, J. A., Jr.; Pelletier, J. *PLoS ONE* **2009**, *4*, e5223.

(8) Gerard, B.; Cencic, R.; Pelletier, J.; Porco, J. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 7831−7834.

(9) El Sous, M.; Khoo, M. L.; Holloway, G.; Owen, D.; Scammells, P. J.; Rizzacasa, M. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 7835−7838.

(10) Adams, T. E.; El Sous, M.; Hawkins, B. C.; Hirner, S.; Holloway, G.; Khoo, M. L.; Owen, D. J.; Savage, G. P.; Scammells, P. J.; Rizzacasa, M. A. *J. Am. Chem. Soc.* **2009**, *131*, 1607−1616.

(11) Kim, S.; Salim, A.; Swanson, S. M.; Kinghorn, A. D. *Anti-Cancer Agents Med. Chem.* **2006**, *6*, 319−345.

(12) Salim, A. A.; Chai, H.-B.; Richman, I.; Riswan, S.; Kardono, L. B. S.; Farnsworth, N. R.; Carcache-Blanco, E. J.; Kinghorn, A. D. *Tetrahedron* **2007**, *63*, 7926−7934.

(13) Seger, C.; Pointinger, S.; Greger, H.; Hofer, O. *Tetrahedron Lett.* **2008**, *49*, 4313−4315.

(14) Govindachari, T. R.; Suresh, G.; Kumari, G. N. K. *Phytochemistry* **1994**, *37*, 1127−1129.

(15) Tokunaga, N.; Sakakibara, N.; Umezawa, T.; Ito, Y.; Fukuda, H.; Sato, Y. *Plant Cell Physiol.* **2005**, *46*, 224−232.

(16) Ding, L.; Pfoh, R.; Ruehl, S.; Qin, S.; Laatsch, H. *J. Nat. Prod.* **2009**, *72*, 99−101.

(17) Witz, P.; Herrmann, H.; Lehn, J. M.; Ourisson, G. *Bull. Soc. Chim. Fr.* **1963**, 1101−1112.

(18) Klinot, J.; Vsetecka, V.; Klinotova, E.; Vystrcil, A. *Collect. Czech. Chem. Commun.* **1986**, *51*, 611−620.

(19) Pointinger, S.; Promdang, S.; Vajrodaya, S.; Pannell, C. M.; Hofer, O.; Mereiter, K.; Greger, H. *Phytochemistry* **2008**, *69*, 2696−2703.

(20) Djerassi, C.; Riniker, R.; Riniker, B. *J. Am. Chem. Soc.* **1956**, *78*, 6362−6377.

(21) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.

(22) Renard, P.; Ernest, I.; Houbion, A.; Art, M.; Le Calvez, H.; Raes, M.; Remacle, J. *Nucleic Acids Res.* **2001**, *29*, e21.

(23) Deng, Y.; Balunas, M. J.; Kim, J.-A.; Lantvit, D. D.; Chin, Y.-W.; Chai, H.-B.; Sugiarso, S.; Kardono, L. B. S.; Fong, H. H. S.; Pezzuto, J. M.; Swanson, S. M.; Carcache-Blanco, E. J.; Kinghorn, A. D. *J. Nat. Prod.* **2009**, *72*, 1165−1169.