Coumarinolignans from the Root of Formosan *Antidesma pentandrum* var. *barbatum*

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Four new coumarinolignans, antidesmanin A (= 7-(1,1-dimethylallyl)-2,3-dihydro-3-(4-hydroxy-3,5-dimethoxyphenyl)-10-methoxy-2-methyl-6*H*-1,4,5-trioxaphenanthren-6-one; **1**), antidesmanin B (= 3,7-bis(1,1-dimethylallyl)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-10-methoxy-6*H*-1,4,5-trioxaphenanthren-6-one; **2**), antidesmanin C (= 2,7-bis-(1,1-dimethylallyl)-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-10-methoxy-6*H*-1,4,5-trioxaphenanthren-6-one; **3**) and antidesmanin D (= 2-(3,4-dihydroxyphenyl)-3,7-bis(1,1-dimethylallyl)-2,3-dihydroxy-10-methoxy-6*H*-1,4,5-trioxaphenanthren-6-one or 3-(3,4-dihydroxyphenyl)-2,7-bis(1,1-dimethylallyl)-2,3-dihydro-10-methoxy-6*H*-1,4,5-trioxaphenanthren-6-one; **4**) have been isolated from the root of the Formosan *Antidesma pentandrum* var. *barbatum*. The structures of these new compounds were elucidated by spectroscopic data. Compounds **1**–**3** exhibited marginal cytotoxicity against MCF-7 (breast) and SF-268 (CNS) cancer cell lines *in vitro*.

Introduction. – *Antidesma pentandrum* MERR. var. *barbatum* (PRESL) MERR. (Euphorbiaceae) is a small shrub distributed throughout the Ryukyus, the northern Philippines, and Taiwan [1], and tannin constituents from the leaves of this plant have been reported [2]. In a screening program of Formosan plants, the MeOH extract of the root of this species was found to exhibit significant cytotoxic activity. Examination of the root of this species led to the isolation of four new coumarinolignans, antidesmanin A–D (**1**–**4**). This paper describes the structural elucidation of these four new compounds by spectral data and the cytotoxicity of the isolates.

Results and Discussion. – Antidesmanin A (**1**) was isolated as an optically inactive colorless oil. Its molecular formula, $C_{26}H_{28}O_8$, was determined by EI-MS (M^+ at m/z 468) and HR-EI-MS (M^+ at 468.1779). The presence of a coumarin moiety was revealed by the UV (328, 235 (sh), 220 nm), IR (1710, 1615, 1580 cm^{-1}), and ^{13}C-NMR data (δ 159.5) [3]. The presence of a phenolic moiety was suggested by the bathochromic shift of the UV spectrum on KOH addition, and by the IR data (3440 cm^{-1}). According to further data, **1** was characterized as 7-(1,1-dimethylallyl)-2,3-dihydro-3-(4-hydroxy-3,5-dimethoxyphenyl)-10-methoxy-2-methyl-6*H*-1,4,5-trioxaphenanthren-6-one[1]), a structure consistent with all ^1H-NMR (*Table 1*) COSY,

[1]) Arbitrary numbering; for systematic names, see *Exper. Part.*

NOESY (*Fig. 1*), ^{13}C-NMR (*Table 2*), DEPT, HMQC, and HMBC (*Fig. 1*) experiments.

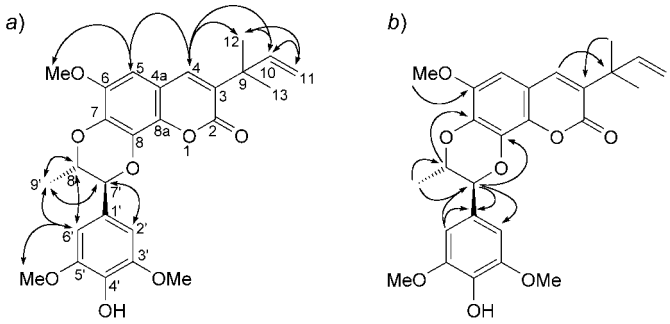

Fig. 1. a) *NOESY Contacts and* b) *key HMBC connectivities of compound* **1**[1])

Table 1. *^{1}H-NMR Data* ($CDCl_3$) *of Compounds* **1**–**4**. Chemical shifts δ in ppm rel. to $SiMe_4$, J in Hz. Arbitrary numbering[1]).

	1[a])	**2**[a])	**3**[a])	**4**[b])
H–C(4)	7.50 (*s*)	7.49 (*s*)	7.46 (*s*)	7.47 (*s*)
H–C(5)	6.51 (*s*)	6.47 (*s*)	6.50 (*s*)	6.46 (*s*)
H–C(10)	6.12 (*dd*, J = 17.4, 10.6)	6.19 (*dd*, J = 17.8, 10.4)	6.17 (*dd*, J = 17.8, 10.2)	6.18 (*dd*, J = 17.5, 11.0)
H_a–C(11)	5.09 (*d*, J = 17.4)	5.09 (*d*, J = 17.8)	5.05 (*d*, J = 17.8)	5.09 (*d*, J = 17.5)
H_b–C(11)	5.10 (*d*, J = 10.6)	5.10 (*d*, J = 10.4)	5.07 (*d*, J = 10.2)	5.09 (*d*, J = 11.0)
Me(12)	1.49 (*s*)	1.49 (*s*)	1.45 (*s*)	1.49 (*s*)
Me(13)	1.49 (*s*)	1.49 (*s*)	1.45 (*s*)	1.49 (*s*)
H–C(2′)	6.61 (*s*)	6.84 (br. *s*)	6.86 (*s*)	6.75–6.85 (*m*)
H–C(5′)		6.87 (br. *s*)	6.86 (*s*)	6.75–6.85 (*m*)
H–C(6′)	6.61 (*s*)	6.87 (br. *s*)	6.86 (*s*)	6.75–6.85 (*m*)
H–C(7′)	4.61 (*d*, J = 7.6)	4.94 (*d*, J = 6.3)	4.99 (*d*, J = 5.6)	4.93 (*d*, J = 5.6)
H–C(8′)	4.21 (*dq*, J = 7.6, 6.4)	4.06 (*d*, J = 6.3)	4.15 (*d*, J = 5.6)	4.02 (*d*, J = 5.6)
Me(9′)	1.29 (*d*, J = 6.4)			
H–C(10′)		5.78 (*dd*, J = 17.8, 10.4)	5.66 (*dd*, J = 17.8, 10.4)	5.76 (*dd*, J = 17.2, 11.0)
H_a–C(11′)		4.87 (*d*, J = 17.8)	4.91 (*d*, J = 17.8)	4.87 (*d*, J = 11.0)
H_b–C(11′)		4.88 (*d*, J = 10.4)	4.92 (*d*, J = 10.4)	4.88 (*d*, J = 17.2)
Me(12′)		0.96 (*s*)	0.99 (*s*)	0.96 (*s*)
Me(13′)		1.19 (*s*)	1.17 (*s*)	1.15 (*s*)
MeO–C(6)	3.88 (*s*)	3.84 (*s*)	3.86 (*s*)	3.84 (*s*)
MeO–C(3′)	3.92 (*s*)	3.86 (*s*)	3.76 (*s*)	
MeO–C(5′)	3.92 (*s*)			
OH–C(4′)	5.62 (*s*)[c])	5.72 (*s*)[c])	5.71 (*s*)[c])	

[a]) At 200 MHz. [b]) At 600 MHz. [c]) Exchangeable with D_2O.

Table 2. *^{13}C-NMR Data* ($CDCl_3$, 50 MHz) *of Compounds* **1**–**3**. Chemical shifts δ in ppm rel. to $SiMe_4$. Arbitrary numbering[1]).

	1	**2**	**3**
C(2)	159.5	159.7	159.7
C(3)	132.7	132.6	132.5
C(4)	138.0	138.1	138.2
C(4a)	111.9	111.9	111.7
C(5)	99.9	99.8	101.2
C(6)	145.6	145.4	145.6
C(7)	136.6	136.0	136.9
C(8)	131.8	132.0	131.6
C(8a)	137.9	137.9	138.3
C(9)	40.5	40.5	40.6
C(10)	145.5	145.6	145.8
C(11)	112.1	112.1	112.2
C(12)	26.0	26.0	26.2
C(13)	26.0	26.0	26.2
C(1′)	127.0	128.8	129.2
C(2′)	104.5	110.9	111.0
C(3′)	147.3	146.6	146.5
C(4′)	135.6	146.5	146.8
C(5′)	147.3	114.4	114.4
C(6′)	104.5	121.8	121.7
C(7′)	81.6	77.3	76.2
C(8′)	74.3	82.6	83.0
C(9′)	17.1	40.9	41.3
C(10′)		142.7	143.1
C(11′)		112.7	113.1
C(12′)		25.1	25.2
C(13′)		23.1	23.2
*Me*O–C(6)	56.2	56.0	56.9
*Me*O–C(3′)	56.5	56.3	56.2
*Me*O–C(5′)	56.5		

The absence of a *d* at δ 6.15–6.35 and the presence of a *s* at δ 7.50 (H–C(4)) in the ^{1}H-NMR spectrum of **1** suggested that H–C(3) was substituted by a 1,1-dimethylallyl group (δ 1.49 (*s*, Me(12), Me(13)), 5.09 (*d*, J = 17.4 Hz, H_a–C(11)), 5.10 (*d*, J = 10.6 Hz, H_b–C(11)), and 6.12 (*dd*, J = 17.4, 10.6 Hz, H–C(10))). The chemical shift of H–C(4) being smaller than δ 7.8 suggested that there is no substituent at C(5) but a proton, appearing at δ 6.51 [3]. The NOESY experiment (*Fig. 1*) showed that H–C(5) at δ 6.51 correlated with H–C(4) at δ 7.50 and a MeO group at δ 3.88 (*s*), and H–C(4) correlated with H–C(5) and the Me groups at δ 1.49 of the 1,1-dimethylallyl group. Thus, the above data supported the assignments of this MeO group at C(6) and the 1,1-dimethylallyl group at C(3). Additionally, the presence of a 4-hydroxy-3,5-dimethoxyphenyl group (δ 3.92 (*s*, MeO–C(3′) and MeO–C(5′)), 5.62 (*s*, exchangeable with D_2O, OH–C(4′)) and 6.61 (*s*, H–C(2′) and H–C(6′))), two vicinal oxymethine protons at δ 4.21 (*dq*, J = 7.6, 6.4 Hz) and 4.61 (*d*, J = 7.6 Hz)) and a Me group at δ 1.29 (*d*, J = 6.4 Hz) in the ^{1}H-NMR spectrum indicated the presence of a phenylpropanoid moiety in **1** as determined by COSY and NOESY experiments (*Fig. 1*). The two oxymethine protons were involved in ether linkages to C(7) and C(8) of the coumarin moiety. The HMBC correlations H–C(8′)/C(7) and H–C(7′)/C(8) confirmed the two ether linkages between C(8′) and C(7) and between C(7′) and C(8). The coupling constants of H–C(8′) and H–C(7′) were J = 7.6 Hz, which indicated that they are in a *trans*-relationship [4][5]. However, in view of the optical inactivity, **1** was concluded to be racemic.

Antidesmanin B (**2**) was isolated as optically inactive colorless oil. Its molecular formula, $C_{29}H_{32}O_7$, was determined by EI-MS (M^+ at m/z 492) and HR-EI-MS (M^+ at

492.2177). The UV, IR, and ^{1}H-NMR spectra (*Table 1*) of **2** were similar to those of **1**. Based on spectral evidences, **2** was characterized as 3,7-bis(1,1-dimethylallyl)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-10-methoxy-6*H*-1,4,5-trioxaphenanthren-6-one[1]), which was further confirmed by COSY, NOESY (*Fig. 2*), ^{13}C-NMR (*Table 2*), DEPT, HMQC, and HMBC (*Fig. 2*) experiments.

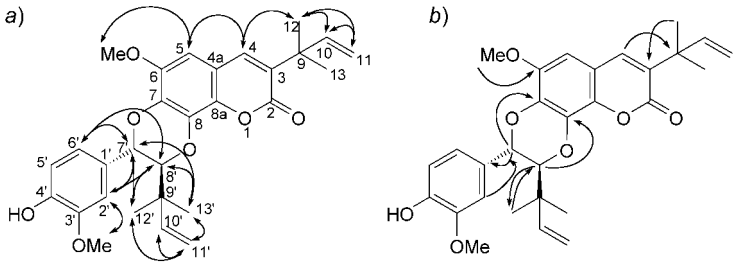

Fig. 2. a) *NOESY Contacts and* b) *key HMBC connectivities of compound* **2**[1])

The ^{1}H-NMR spectrum of **2** confirmed the presence of a coumarinolignan moiety. Compared to **1**, **2** showed the signals of an additional 1,1-dimethylallyl group (δ 0.96 (*s*, Me(12′)), 1.19 (*s*, Me(13′)), 4.87 (*d*, J = 17.8 Hz, H$_a$–C(11′)), 4.88 (*d*, J = 10.4 Hz, H$_b$–C(11′)), and 5.78 (*dd*, J = 17.8, 10.4 Hz, H–C(10′))) instead of that of Me–C(8′) of **1**, and those of a 4-hydroxy-3-methoxyphenyl group (δ 6.84 (br. *s*, H–C(2′)), 6.87 (br. *s*, H–C(5′), H–C(6′)), 3.86 (*s*, MeO–C(3′)), and 5.72 (*s*, OH–C(4′), exchangeable with D$_2$O)) instead of the 4-hydroxy-3,5-dimethoxyphenyl group of **1**. The HMBC correlations H–C(7′)/C(7) and H–C(8′)/C(8) confirmed the two ether linkages between C(7′) and C(7) and between C(8′) and C(8). The coupling constant (J = 6.3 Hz) of the two vicinal oxymethine protons at the coumarinolignan moiety indicated that they are *trans*-positioned [4][5]. Because of the optical inactivity, **2** was concluded to be racemic.

Antidesmanin C (**3**) was also isolated as optically inactive colorless oil. Its molecular formula, $C_{29}H_{32}O_7$, was determined by EI-MS (M^+ at m/z 492) and HR-EI-MS (M^+ at 492.2144). The UV, IR and ^{1}H-NMR spectra (*Table 1*) of **3** were similar to those of **2**, and **3** was found to be a positional isomer of **2**. As shown for **1** and **2**, the HMBC spectrum of **3** (*Fig. 3*), confirmed the position of the two ether linkages, and the coupling constant (J = 5.6 Hz) of H–C(8′) and H–C(7′) for their *trans*-relationship [4][5]. Because of the optical inactivity, **3** was considered to be racemic. According to these evidences, **3** was characterized as 2,7-bis(1,1-dimethylallyl)-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-10-methoxy-6*H*-1,4,5-trioxaphenanthren-6-one[1]), which was further confirmed by COSY, NOESY (*Fig. 3*), ^{13}C-NMR (*Table 2*), DEPT, HMQC, and HMBC (*Fig. 3*) experiments.

Antidesmanin D (**4**) was also isolated as a colorless oil, which easily decomposed. Its molecular formula, $C_{28}H_{30}O_7$, was determined by EI-MS (M^+ at m/z 478). The ^{1}H-NMR spectrum of **4** (*Table 1*) was similar to that of **2**, except for the presence of only one MeO group *s* at δ 3.84 (MeO–C(6)) and three aromatic protons (δ 6.75–6.85), which indicated the presence of a 3,4-dihydroxyphenyl group. The location of the 3,4-dihydroxyphenyl group and 1,1-dimethylallyl group of **4** could not be established due to the small quantity available for study and the absence of an HMBC spectrum; therefore, we propose two possible structures for **4** (*Fig. 4*), *i.e.*, 2-(3,4-dihydroxyphenyl)-3,7-bis(1,1-dimethylallyl)-2,3-dihydro-10-methoxy-6*H*-1,4,5-trioxaphenanthren-

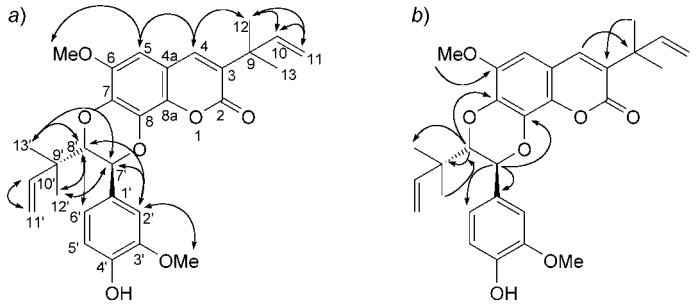

Fig. 3. a) *NOESY Contacts and* b) *key HMBC connectivities of compound* **3**[1])

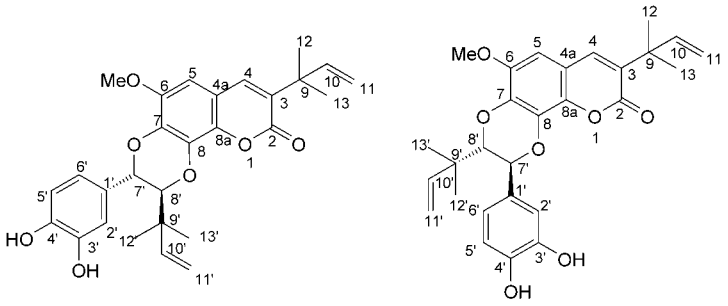

Fig. 4. *Possible structures of compound* **4**[1])

6-one[1]) or 3-(3,4-dihydroxyphenyl)-2,7-bis(1,1-dimethylallyl)-2,3-dihydro-10-methoxy-6*H*-1,4,5-trioxaphenanthren-6-one[1]).

Coumarinolignans have been isolated from Aceraceae [6][7], Sapindaceae [8][9], Capparidaceae [3][10–12], Thymelaeaceae [13–17], Burseraceae [18], Ranunculaceae [19], Chenopodiaceae [20], Asclepiadaceae [21], Malvaceae [22], Verbenaceae [23], and Euphorbiaceae [24][25], but the 1,1-dimethylallyl substituent in the coumarinolignan moiety like compounds **1**–**4** has never been found in nature.

Compounds **1**–**3** were evaluated for their *in vitro* effects on the growth of three human cancer cell lines: MCF-7 (breast), NCI-H460 (lung), and SF-268 (CNS). At 20 μg/ml, antidesmanin A (**1**) exhibited a 25% cell-growth effect on the cancer cell line MCF-7, antidesmanin B (**2**) showed a 7% cell-growth effect on the cancer cell line MCF-7 and a 10% cell-growth effect on the cancer cell line SF-268, and antidesmanin C (**3**) showed a 28% cell-growth effect on the cancer cell line MCF-7 and a 20% cell-growth effect on the cancer cell line SF-268. However, these compounds were ineffective against the NCI-H460 cell line.

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Experimental Part

General. TLC: silica gel *60* F_{254} precoated plates (*Merck*). Column chromatography (CC): silica gel *60* (*Merck* 70–230 mesh, 230–400 mesh, ASTM). M.p.: *Yanaco* micro-melting-point apparatus; uncorrected. Optical rotation: *Jasco DIP-370* polarimeter; in $CHCl_3$. UV Spectra: *Jasco UV-240* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Perkin-Elmer 2000* FT-IR spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H-, ^{13}C-, and 2D-NMR Spectra: *Jeol GSX-600*, *Varian Unity-Plus-400*, and *Varian Gemini-200* spectrometers; δ in ppm rel. to $SiMe_4$, *J* in Hz. EI-MS: *VG Biotech-Quatro-5022* spectrometer; *m*/*z* (rel. %). HR-EI-MS: *Jeol SX-102A* mass spectrometer.

Plant Material. The root of *Antidesma pentandrum* var. *barbatum* was collected from Lai-I, Pingtung County, Taiwan, in September 2002, and identified by Dr. *I. S. Chen*. A voucher specimen (Chen 6109) was deposited in the Herbarium of the School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.

Extraction and Isolation. Air-dried root (8.5 kg) of *A. pentandrum* var. *barbatum* was extracted repeatedly with cold MeOH. After evaporation, the extract was partitioned into hexane (42.6 g), AcOEt (19.5 g), and H_2O-soluble (558 g) parts. Because of the same TLC traces, the hexane- and AcOEt-soluble fractions were combined (*Fr. A*) and submitted to CC (silica gel, $CHCl_3$ and $CHCl_3$/MeOH of increasing polarity): *Fr. A.1–A.8.* Repeated purification of *Fr. A.3* ($CHCl_3$/MeOH 17:3; 24.5 g) by CC (silica gel, $CHCl_3$/AcOEt) gave **1** (9.6 mg), **2** (101.1 mg), and **3** (10.6 mg). *Fr. A.5* ($CHCl_3$/MeOH 4:1; 2.1 g) was submitted to CC (silica gel, $CHCl_3$ and $CHCl_3$/MeOH of increasing polarity): *Fr. A.5.1–A.5.8. Fr. A.5.1* (72.0 mg) was further purified by prep. TLC ($CHCl_3$/acetone/MeOH 38:1:1): **4** (1.5 mg).

*7-(1,1-Dimethylallyl)-2,3-dihydro-3-(4-hydroxy-3,5-dimethoxyphenyl)-10-methoxy-2-methyl-6*H*-1,4,5-trioxaphenanthren-6-one* (=*(2*RS,*3*RS*)-2-(1,1-Dimethylprop-2-enyl)-2,3-dihydro-2-(4-hydroxy-3,5-dimethoxyphenyl)-5-methoxy-3-methyl-9*H*-pyrano[3,2-*h*]-1,4-benzodioxin-9-one = Antidesmanin A*; **1**): Colorless oil. *Gibbs* test: negative. $[\alpha]_D^{21} = 0$ ($c = 0.48$, $CHCl_3$). UV (MeOH): 328 (4.67), 235 (sh, 4.91), 220 (5.01). UV (MeOH + KOH): 346 (4.66), 262 (4.70), 222 (5.02). IR (KBr): 3440 (OH), 1710 (C=O), 1615, 1580, 1520, 1500, 1460, 1400, 1350, 1310, 1220, 1140, 1110. ^{1}H-NMR ($CDCl_3$, 200 MHz): *Table 1*. ^{13}C-NMR ($CDCl_3$, 50 MHz): *Table 2*. EI-MS: 469 (69), 468 (100, M^+), 303 (20), 302 (99), 279 (49), 265 (28). HR-EI-MS: 468.1779 ($C_{26}H_{28}O_8^+$; calc. 468.1784).

*3,7-Bis(1,1-dimethylallyl)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-10-methoxy-6*H*-1,4,5-trioxaphenanthren-6-one* (=*(2*RS,*3*RS*)-2,8-Bis(1,1-dimethylprop-2-enyl)-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-5-methoxy-9*H*-pyrano[3,2-*h*]-1,4-benzodioxin-9-one = Antidesmanin B*; **2**): Yellowish oil. *Gibbs* test: negative. $[\alpha]_D^{21} = 0$ ($c = 5.05$, $CHCl_3$). UV (MeOH): 331 (4.77), 289 (sh, 4.50), 232 (5.00), 223 (5.00). UV (MeOH + KOH): 340 (4.78), 305 (sh, 4.68), 258 (4.83), 222 (5.00). IR (KBr): 3430 (OH), 1710 (C=O), 1615, 1580, 1520, 1460, 1400, 1300, 1280, 1235, 1200, 1140, 1070. ^{1}H-NMR ($CDCl_3$, 200 MHz): *Table 1*. ^{13}C-NMR ($CDCl_3$, 50 MHz): *Table 2*. EI-MS: 492 (100, M^+), 425 (13), 424 (51), 423 (89), 395 (23), 394 (77), 363 (16), 356 (46), 287 (58), 277 (11), 276 (52), 261 (26). HR-EI-MS: 492.2177 ($C_{29}H_{32}O_7^+$; calc. 492.2148).

*2,7-Bis(1,1-dimethylallyl)-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-10-methoxy-6*H*-1,4,5-trioxaphenanthren-6-one* (=*(2*RS,*3*RS*)-3,8-Bis(1,1-dimethylprop-2-enyl)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-5-methoxy-9*H*-pyrano[3,2-*h*]-1,4-benzodioxin-9-one = Antidesmanin C*; **3**): Colorless oil. *Gibbs* test: negative. $[\alpha]_D^{25} = 0$ ($c = 0.53$, $CHCl_3$). UV (MeOH): 332 (4.21), 288 (3.98), 235 (sh, 4.50), 219 (4.60). UV (MeOH + KOH): 348 (4.24), 302 (4.08), 258 (4.32), 219 (4.61). IR (KBr): 3420 (OH), 1710 (C=O), 1615, 1580, 1515, 1460, 1400, 1310, 1270, 1235, 1200, 1135, 1070. ^{1}H-NMR ($CDCl_3$, 200 MHz): *Table 1*. ^{13}C-NMR ($CDCl_3$, 50 MHz): *Table 2*. EI-MS: 493 (31), 492 (100, M^+), 425 (23), 424 (52), 423 (23), 422 (21), 396 (18), 395 (66), 363 (16), 327 (16), 287 (29), 276 (21), 260 (13). HR-EI-MS: 492.2144 ($C_{29}H_{32}O_7^+$; calc. 492.2148).

*2-(3,4-Dihydroxyphenyl)-3,7-bis(1,1-dimethylallyl)-2,3-dihydro-10-methoxy-6*H*-1,4,5-trioxaphenanthren-6-one or 3-(3,4-Dihydroxyphenyl)-2,7-bis(1,1-dimethylallyl)-2,3-dihydro-10-methoxy-6*H*-1,4,5-trioxaphenanthren-6-one* (=*(2*RS,3RS*)-3-(3,4-Dihydroxyphenyl)-2,8-bis(1,1-dimethylprop-2-enyl)-2,3-dihydro-5-methoxy-9*H*-pyrano[3,2-*h*]-1,4-benzodioxin-9-one or (2*RS,*3*RS*)-2-(3,4-Dihydroxyphenyl)-3,8-bis(1,1-dimethylprop-2-enyl)-2,3-dihydro-5-methoxy-9*H*-pyrano[3,2-*h*]-1,4-benzodioxin-9-one = Antidesmanin D*; **4**): Colorless oil. ^{1}H-NMR ($CDCl_3$, 600 MHz): *Table 1*. EI-MS: 478 (2, M^+), 407 (54), 379 (20), 355 (18), 276 (71), 261 (39), 233 (82), 221 (100), 189 (63), 161(81), 128 (81), 115 (70).

Cytotoxicity Assay. MCF-7 cells (human breast adenocarcinoma), NCI-H460 cells (non-small-cell lung cancer) and SF-268 cells (glioblastoma cells) were cultured in *Dulbecco*'s modified *Eagle*'s medium supplemented with 10% fetal calf serum and nonessential amino acid (*Life Technologies*, *Inc.*) and maintained at 37° in a humidified incubator with 5% CO_2.

Human cancer cells were seeded in 96-well microtiter plates at a density of 6500, 2500 and 7500 cells/well in 100 µl of culture medium for MCF-7, NCI-H460, and SF-268, respectively. After an overnight adaptation period, 20 µg/ml (final concentration) of test compounds in serum-free medium were added to individual wells. Cells were treated with test compounds for 3 days. Cell viability was determined by the 5-[3-(carboxymethoxy)-phenyl]-2-(4,5-dimethylthiazolyl)-3-(4-sulfophenyl)tetrazolium salt (MTS) reduction assay [26–28]. Actinomycin D (10 µM, final concentration) and DMSO (0.1%, final concentration) were used as positive and vehicle controls, resp. Results were expressed as % of the DMSO control.

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