Cytotoxic Phenanthrenequinones and 9,10-Dihydrophenanthrenes from *Calanthe arisanensis*

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Two new phenanthrenequinones, calanquinones B and C (**2** and **3**) and four new 9,10-dihydrophenanthrenes, calanhydroquinones A−C (**4−6**) and calanphenanthrene A (**7**), along with five known compounds (**1** and **8−11**), were isolated from an EtOAc-soluble extract of *Calanthe arisanensis* through bioassay-guided fractionation. Their structures were identified from spectroscopic data, and the compounds were tested for in vitro cytotoxic activity against human lung (A549), prostate (PC-3 and DU145), colon (HCT-8), breast (MCF-7), nasopharyngeal (KB), and vincristine-resistant nasopharyngeal (KBVIN) cancer cell lines. Compound **1** showed the highest potency (EC$_{50}$ < 0.5 μg/mL) against all seven cancer cell lines, with the greatest activity against breast cancer MCF-7 cells (EC$_{50}$ < 0.02 μg/mL). Generally, except for **7**, compounds **2−11** also showed significant cytotoxic activity (EC$_{50}$ < 4 μg/mL) against some cell lines (especially PC-3 and MCF-7) in the panel.

The genus *Calanthe* in the Orchidaceae family contains terrestrial perennial herbs that are widely distributed from tropical Africa and Madagascar to tropical and subtropical Asia, China, Japan, southward through Malaysia and Indonesia, to the Pacific Islands and Australia. This genus includes more than 150 species, but only 19 are found in Taiwan. Among them, *C. arisanensis* Hayata (Orchidaceae) is endemic to Taiwan and grows in forests from 1000 to 2000 m throughout the island.[1] No phytochemical study of this plant has been reported to date. During cytotoxicity screening of extracts of Formosan plants, we found that an EtOAc extract of *C. arisanensis* was active against various human cancer cell lines, with IC$_{50}$ < 20 μg/mL. Bioassay-directed chromatographic fractionation of this extract led to isolation of two new phenanthrenequinones [calanquinones B and C (**2**, **3**)] and four new 9,10-dihydrophenanthrenes [calanhydroquinones A−C (**4−6**) and calanphenanthrene A (**7**)], as well as five known compounds (**1** and **8−11**).[2−6] Compounds **1−11** were screened for cytotoxic activity against human lung (A549), prostate (PC-3 and DU145), colon (HCT-8), breast (MCF-7), nasopharyngeal (KB), and vincristine-resistant nasopharyngeal (KBVIN) cancer cell lines.

Results and Discussion

The cytotoxic MeOH extract of dry roots of *C. arisanensis* was partitioned between EtOAc and water to give an active EtOAc extract. Column chromatography on silica gel eluting with CHCl$_3$−MeOH gave 13 fractions. Chromatographic fractionation of the active subfractions provided two new phenanthrenequinones (**2**, **3**), four new 9,10-dihydrophenanthrenes (**4−7**), and five known compounds (**1** and **8−11**).

HRESIMS of **2** showed an [M − H]$^-$ ion at *m/z* 313.0690 (C$_{17}$H$_{13}$O$_6$), indicating 11 degrees of unsaturation. The IR spectrum showed absorptions for OH (3380 cm^{-1}), carbonyl (1682 and 1640 cm^{-1}), and aromatic (1616, 1478, 1427, and 842 cm^{-1}) functional
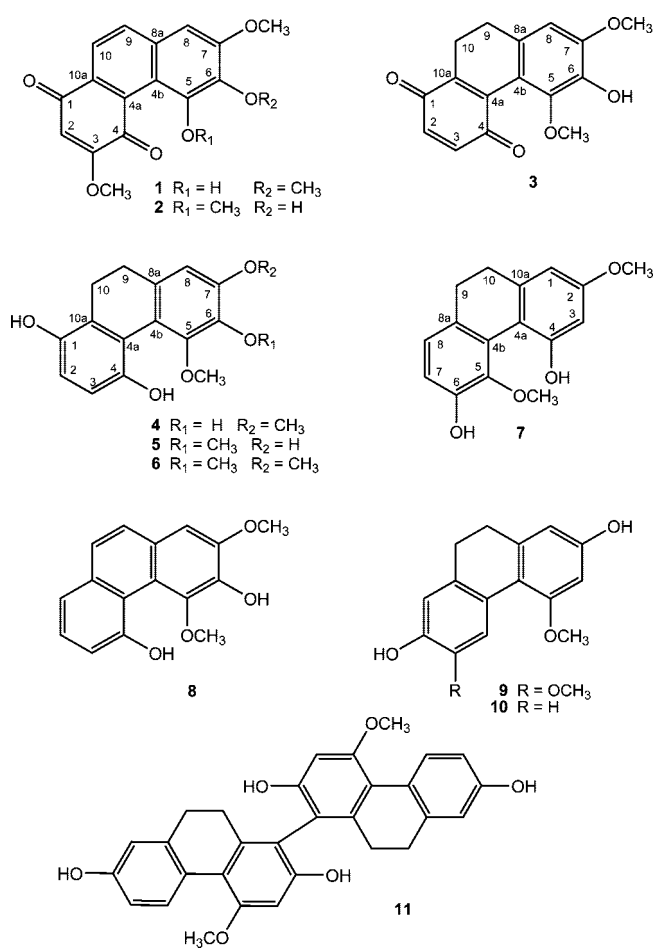
groups. UV absorptions at 249, 296, and 406 nm also indicated an aromatic system. Seventeen carbon signals, including three methoxy, four methine, and 10 quaternary carbons, were observed in the NMR spectra of **2** (Tables 1 and 2). Among the 10 quaternary carbons, two were identified as carbonyl carbons on the basis of chemical shifts at δ 181.4 and 184.8. Therefore, the data supported the presence of two carbonyls, six olefins, and three ring moieties to fulfill the 11 degrees of unsaturation, and compound **2** was

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Table 1. ^{1}H NMR Data of Compounds **1**−**7** (300 MHz, δ in ppm, J in Hz) (**1**, **2** in CDCl$_3$; **3**−**7** in C$_5$D$_5$N)

proton	1	2	3	4	5	6	7
1							6.70 d (2.4)
2	6.15 s	6.04 s	6.77 d (9.9)	7.27 d (8.7)	7.23 d (8.7)	7.26 d (8.5)	
3			6.94 d (9.9)	7.29 d (8.7)	7.29 d (8.7)	7.29 d (8.5)	6.94 d (2.4)
4							
4a							
4b							
5							
6							
7							7.18 d (8.0)
8	6.86 s	6.98 s	6.67 s	6.89 s	7.10 s	6.87 s	7.08 d (8.0)
8a							
9	8.05 d (8.7)	7.90 d (8.4)	a, b-2.55 m	a, b-2.71 m	a, b-2.67 m	a, b-2.70 m	a, b-2.62 m
10	8.10 d (8.7)	7.97 d (8.4)	a, b-2.56 m	a, b-3.21 m	a, b-3.17 m	a, b-3.18 m	a, b-2.73 m
10a							
OCH$_3$	3.96 s (C3)	3.94 s (C3)	4.26 s (C5)	3.82 s (C5)	3.77 s (C5)	3.76 s (C5)	3.75 s (C2)
	4.02 s (C6)	3.97 s (C5)	3.77 s (C7)	3.84 s (C7)	3.88 s (C6)	3.90 s (C6)	3.77 s (C5)
	4.01 s (C7)	4.06 s (C7)				3.83 s (C7)	
−OH			11.20 s	9.06 s	8.97 s	8.84 s	
				11.00 br	11.40 br	11.04 s	

Table 2. ^{13}C NMR Data of Compounds **1**−**7** (300 MHz, δ in ppm) (**1**, **2** in CDCl$_3$; **3**−**7** in C$_5$D$_5$N)

carbon	1	2	3	4	5	6	7
1	184.7	184.8	185.7	148.7	147.9	147.6	107.2
2	107.4	106.4	135.6	116.9	116.1	116.7	160.9
3	161.7	162.9	137.4	118.3	117.6	118.0	103.0
4	186.2	181.4	186.2	148.1	147.2	147.6	156.5
4a	128.3	130.6	141.7	123.0	122.2	122.1	114.0
4b	118.7	119.8	117.1	120.5	118.2	120.2	127.1
5	148.3	141.9	147.7	145.3	149.5	148.2	143.7
6	140.4	140.8	139.7	140.2	140.3	141.5	150.6
7	155.2	150.2	151.5	149.0	151.3	152.8	115.3
8	101.4	102.4	106.6	109.3	113.5	109.4	124.9
8a	135.1	132.0	131.2	131.8	137.2	137.1	131.5
9	137.1	132.8	28.3	31.0	30.5	30.9	30.3
10	122.0	120.2	20.9	24.1	23.2	23.3	32.3
10a	133.0	131.6	140.4	127.6	126.6	127.0	143.1
−OCH$_3$	57.1 (C3)	56.5 (C3)	60.6 (C5)	61.9 (C5)	61.8 (C5)	62.3 (C5)	55.2 (C2)
	61.0 (C6)	56.3 (C5)	56.1 (C7)	56.6 (C7)	60.7 (C6)	61.1 (C6)	61.3 (C5)
	56.2 (C7)	60.3 (C7)				56.0 (C7)	

postulated to be a phenanthrenedione or anthracenedione.[2,7] Compounds **1** and **2** had different TLC R_f values (0.4 and 0.2, *n*-hexane−EtOAc, 1:1) and HPLC retention times (t_R 7.5 and 5.7 min), but the same molecular formula, C$_{17}$H$_{14}$O$_6$.[2] Likewise, the two compounds had almost identical spectroscopic data, with the only dissimilarities suggesting different placements of one OCH$_3$ group and one OH group.[2] In the HMBC spectra of **2**, methoxy protons at δ 3.97 and 4.06 showed ^3J interactions with carbons at δ 141.9 (C-5) and 150.2 (C-7), respectively, and the olefinic proton at δ 6.98 exhibited a ^2J interaction with a carbon at δ 150.2 (C-7) and ^3J interactions with carbons at δ 119.8 (C-4b), 140.8 (C-6), and 132.8 (C-9). These correlations led to assignment of an OCH$_3$ group at C-5 (δ 141.9) and an OH group at C-6 (δ 140.8) rather than the reverse (C-5 OH, C-6 OCH$_3$), as found in **1**.[2] The presence of NOESY correlations between δ 6.04 (H-2)/3.94 (3-OMe) and δ 6.98 (H-8)/4.06 (7-OMe) and the absence of correlation between δ 3.97 (5-OMe)/4.06 (7-OMe) suggested that the three OCH$_3$ groups were located at C-3, C-5, and C-7. The new compound **2** was therefore identified as a positional isomer, 6-hydroxy-3,5,7-trimethoxy-1,4-phenanthrenequinone, of **1** and has been named calanquinone B (**2**).

An [M + H]$^+$ ion at m/z 287.0922 (C$_{16}$H$_{15}$O$_5$) was found in the HRESIMS of **3**. IR absorptions at 3386 and 1646 cm^{-1} supported the presence of OH and carbonyl groups, respectively. Other IR absorptions at 1607, 1560, 1466, and 835 cm^{-1} as well as UV absorptions at 218, 273, and 322 nm indicated an aromatic system. In the 1D NMR spectra (Tables 1 and 2), 16 carbon signals were observed, which were consistent with two methoxy, two methylene, three methine, and nine quaternary carbons, including two carbonyl

carbons (δ 185.7 and 186.2). 1D NMR and HSQC data indicated the presence of two methoxy groups [δ_H 3.77 and 4.26 (δ_C 56.1 and 60.6)], two methylene groups [δ_H 2.55 (2H, m, δ_C 28.3) and 2.56 (2H, m, δ_C 20.9)], and three olefinic protons [δ_H 6.67 (s, δ_C 106.6), 6.77 (d, J = 9.9 Hz, δ_C 135.6), and 6.94 (d, J = 9.9 Hz, δ_C 137.4)]. On the basis of HMBC [δ_H 6.67 (H-8)/δ_C 28.3 (C-9), 117.1 (C-4b), 139.7 (C-6), and 151.5 (C-7); δ_H 3.77 (7-OMe)/δ_C 151.5 (C-7); and δ_H 4.26 (5-OMe)/δ_C 147.7 (C-5)] and NOESY correlations [δ 2.56 (H-10)/2.55 (H-9), δ 2.55 (H-9)/6.67 (H-8), δ 6.67 (H-8)/3.77 (7-OMe), and δ 6.77 (H-2)/6.94 (H-3)], the OCH$_3$ groups were assigned at C-5 (δ 147.7) and C-7 (δ 151.5). The structure of **3**, calanquinone C, was elucidated as 6-hydroxy-5,7-dimethoxy-9,10-dihydro-1,4-phenanthrenequinone.

Compound **4** contained OH (3261 cm^{-1}) and aromatic (1613, 1561, 1462, and 801 cm^{-1}) groups, as indicated by the corresponding absorptions in its IR spectrum. HRESIMS showed an [M − H]$^-$ ion at m/z 287.0896 (C$_{16}$H$_{15}$O$_5$). ^{1}H and ^{13}C NMR data (Tables 1 and 2) supported 16 carbons, including two methoxy, two methylene, three methine, and nine quaternary. 1D NMR and HSQC data indicated the presence of OCH$_3$ groups at δ_H 3.82 and 3.84 (δ_C 61.9 and 56.6), two methylene groups at δ_H 2.71 (2H, m, δ_C 31.0) and 3.21 (2H, m, δ_C 24.1), and three olefinic protons at δ_H 6.89 (s, δ_C 109.3), 7.27 (d, J = 8.7 Hz, δ_C 116.9), and 7.29 (d, J = 8.7 Hz, δ_C 118.3). According to HMBC [δ_H 6.89 (H-8)/δ_C 31.0 (C-9), 120.5 (C-4b), 140.2 (C-6), and 149.0 (C-7); δ_H 3.82 (5-OMe)/δ_C 145.3 (C-5); δ_H 3.84 (7-OMe)/δ_C 149.0 (C-7); and δ_H 9.06 (4-OH)/δ_C 118.3 (C-3) and 148.1 (C-4)] and NOESY [δ 2.71 (H-9)/3.21 (H-10) and 6.89 (H-8), δ 6.89 (H-8)/3.84 (7-OMe), δ 7.27 (H-2)/7.29 (H-3), and δ 9.06 (4-OH)/3.82 (5-OMe)] correlations,

Table 3. Cytotoxicity Data for Compounds 1−11

compound	EC$_{50}$ (μg/mL)/cell line						
	A549	PC-3	DU145	HCT-8	MCF-7	KB	KBVIN
1	0.19	0.16	0.34	0.20	<0.02	0.32	0.45
2	4.54	1.55	6.45	4.56	1.81	6.46	13.28
3	3.58	2.07	4.34	4.14	2.12	2.97	4.68
4	3.58	2.52	5.84	4.57	2.31	3.47	5.37
5	4.07	2.62	5.46	5.16	3.28	3.75	5.24
6	2.36	0.85	3.78	3.96	1.70	2.86	4.73
7	7.71	7.06	13.21	6.73	6.52	11.80	5.48
8	5.06	3.38	8.29	5.26	3.26	5.63	6.99
9	4.24	2.67	5.38	4.55	2.59	3.90	5.84
10	5.05	2.82	4.79	3.84	1.80	3.40	7.92
11	4.61	5.37	4.11	4.60	3.50	4.40	3.96
paclitaxel	<0.005	0.0097	<0.005	0.21	0.0072	<0.005	2.16

the OCH$_3$ groups were assigned at C-5 (δ 145.3) and C-7 (δ 149.0). Compound **4**, calanhydroquinone A, was identified as 1,4,6-trihydroxy-5,7-dimethoxy-9,10-dihydrophenanthrene. The 1,4-hydroquinone group of **4** may arise from reduction of the 1,4-quinone group of **3** in the plant.

Compound **5** showed an [M − H]$^-$ ion at *m/z* 287.0902 (C$_{16}$H$_{15}$O$_5$) in the HRESIMS. The mass and NMR data were similar to those of **4**. HMBC correlations [H-8/C-9, C-4b, C-6, and C-7; 5-OMe/C-5; and 6-OMe/C-6] and NOESY [H-9/H-10 and H-8, and H-2/H-3] correlations supported assignment of OCH$_3$ groups at C-5 and C-6. Compound **5** has been named calanhydroquinone B, and its structure was elucidated as 1,4,7-trihydroxy-5,6-dimethoxy-9,10-dihydrophenanthrene, an isomer of **4**.

The molecular formula of **6** was deduced as C$_{17}$H$_{18}$O$_5$ due to the appearance of an [M − H]$^-$ ion at *m/z* 301.1051 in the HRESIMS. Compounds **4**, **5**, and **6** had similar spectroscopic data, except that **6** had an additional OCH$_3$ group in comparison with **4** and **5**. On the basis of HMBC [H-8/C-9, C-4b, C-8a, C-6, and C-7; 5-OMe/C-5; 6-OMe/C-6; and 7-OMe/C-7] and NOESY [H-9/H-10 and H-8; H-8/7-OMe; H-2/H-3; and 4-OH/5-OMe] data, the OCH$_3$ groups were placed at C-5, C-6, and C-7. Compound **6** was identified as 1,4-dihydroxy-5,6,7-trimethoxy-9,10-dihydrophenanthrene and has been named calanhydroquinone C (**6**).

Compounds **4**−**6**, which are 9,10-dihydrophenanthrene derivatives, contain 1,4-hydroquinone groups that likely occur from reduction of 1,4-phenanthrenequinones. Thus, we have isolated and reported herein the 9,10-dihydrophenanthrene-1,4-hydroquinone skeleton for the first time.

Compound **7** has the molecular formula C$_{16}$H$_{16}$O$_4$, as established by HRESIMS (*m/z* 271.0984 [M − H]$^-$). Its IR spectrum showed absorptions attributable to OH (3409 and 3227 cm^{-1}) and aromatic rings (1615, 1480, 1450, 1428, and 844 cm^{-1}). UV absorptions at 219, 274, and 307 nm indicated an aromatic system. 1D NMR and HSQC indicated the presence of OCH$_3$ groups at δ_H 3.75 and 3.77 (δ_C 55.2 and 61.3), two methylene groups at δ_H 2.62 (2H, m, δ_C 30.3) and 2.73 (2H, m, δ_C 32.3), and two pairs of aromatic protons, including one *meta*-coupling at δ_H 6.70 (d, *J* = 2.4 Hz, δ_C 107.2) and 6.94 (d, *J* = 2.4 Hz, δ_C 103.0) as well as one *ortho*-coupling at δ_H 7.08 (d, *J* = 8.0 Hz, δ_C 124.9) and 7.18 (d, *J* = 8.0 Hz, δ_C 115.3). On the basis of HMBC correlations [δ_H 6.70 (H-1)/δ_C 32.3 (C-10), 114.0 (C-4a), 103.0 (C-3), and 160.9 (C-2); δ_H 6.94 (H-3)/δ_C 107.2 (C-1), 114.0 (C-4a), 156.5 (C-4), and 160.9 (C-2); δ_H 7.08 (H-8)/δ_C 127.1 (C-4b), 150.6 (C-6), and 30.3 (C-9); δ_H 7.18 (H-7)/δ_C 131.5 (C-8a), 143.7 (C-5), and 150.6 (C-6); δ_H 3.75 (2-OMe)/δ_C 160.6 (C-2); and δ_H 3.77 (5-OMe)/δ_C 143.7 (C-5)] as well as NOESY correlations [δ 2.62 (H-9)/2.73 (H-10) and 7.08 (H-8); δ 6.70 (H-1)/2.73 (H-10) and 3.75 (2-OMe); δ 6.94 (H-3)/3.75 (2-OMe); and δ 7.08 (H-8)/7.18 (H-7)], the OCH$_3$ groups were assigned at C-2 (δ 160.9) and C-5 (δ 143.7). Compound **7** was elucidated as 4,6-dihydroxy-2,5-dimethoxy-9,10-dihydrophenanthrene and has been named calanphenanthrene A (**7**).

Compounds **1**−**11** were screened in an in vitro cytotoxicity assay, with paclitaxel used as a positive control (Table 3). By far,

compound **1** exhibited the highest potency (IC$_{50}$ 0.02−0.45 μg/mL) against all seven tested cancer cell lines, with its greatest potency against the MCF-7 breast cancer cells (EC$_{50}$ < 0.02 μg/mL). These results were particularly interesting because the structures of many of these compounds are quite similar. For example, compounds **1** (5-OH, 6-OCH$_3$) and **2** (5-OCH$_3$, 6-OH) simply have reversed placements of the OH and one OCH$_3$ group, but **2** was much less potent than **1** and showed significant activity only against PC-3 and MCF-7 cell lines. Compound **3** has the same structure as **2**, with the exception of an additional OCH$_3$ group at C-3. These two compounds showed comparable potencies against most cell lines, with **3** being slightly more potent against A549 and KB cells. Compound **4**, which is the reduced 9,10-dihydrophenanthrene analogue of **2**, showed potency comparable to **3**. Among the three 9,10-dihydrophenanthrene compounds (**4**−**6**), compound **6** showed the highest potency with significant or marginal activity against all seven cell lines. Structurally, it varied from the other two compounds in the number of OCH$_3$ groups (three in **6**, two in **4** and **5**). Compounds **7**−**11**, which do not contain a 1,4-hydroquinone, showed no significant activity or were active only against PC-3 and MCF-7 cell lines, which were the most sensitive lines in this cell panel to this compound type. Overall, our studies identified **1** as a promising lead compound and *C. arisanensis* as a promising plant source of new agents for cancer chemotherapy.

Phenanthrenes are an uncommon class of aromatic metabolites presumably formed by oxidative coupling of the aromatic rings of stilbene precursors and have been reported from higher plants, mainly in the Orchidaceae family. Most natural phenanthrenes occur in monomeric form, and their great structural diversity stems from the number (3 to 6) and position (usually on C-2, C-3, C-5, C-6, or C-7) of their oxygen functions (OH or OCH$_3$) on 9,10-dihydro or dehydro skeletons. Nearly 40 biphenanthrenes, mostly 1−1′ linked, and only one triphenanthene have been reported to date in nature.[9] Phenanthraquinones are monomeric phenanthrenes. Calanquinone A (**1**) is related in structure to other naturally occurring phenanthrenequinones, including denbinobin (lacking the OCH$_3$ group at C-6),[10,11] sphenone A (lacking the C-5 OH group), cymbinodin A (lacking the two OCH$_3$ groups at C-6 and C-7), and annoquinone A (lacking any substituents at C-5, C-6, and C-7).[2,11] In prior studies, denbinobin (A549, EC$_{50}$ 1.3 μg/mL), sphenone A, and annoquinone A (KB, EC$_{50}$ 2.7 and 0.16 μg/mL, respectively) showed significant cytotoxic activity.[2,10] The structurally similar dimeric phenanthrene **11** (9,10-dihydro-) and cirrhopetalanthrin (9,10-dehydro-) both showed cytotoxicity against A549, HCT-8, and MCF-7 cancer cell lines [EC$_{50}$ 9.6 and 17.8, 9.5 and 11.2, 7.3 and 12.5 μM, respectively].[9] Many phenanthrene-containing plants have been used in traditional medicine throughout the world, and phenanthrenes have been identified as their active constituents from phytochemical−pharmacological investigations. According to our studies, the phenanthrenes are a promising group of active natural compounds. Their biological mechanisms and structure−activity relationships have been reported only rarely, and we believe they merit future investigation.

Experimental Section

General Experimental Procedures. Melting points were determined on a Mel-Temp 3.0 instrument (Barnstead International) without correction. UV spectra were measured on a Shimadzu UV-1700 PharmaSpec UV/vis spectrophotometer. IR spectra were recorded on a IRPrestige-21 Shimadzu FT-IR spectrophotometer. NMR spectra were recorded on Varian Gemini 2000 300 HMz and Inova 500 MHz instruments. Chemical shift (δ) values are in ppm (parts per million) with CDCl$_3$ and C$_5$D$_5$N as internal standards, and coupling constants (*J*) are in Hz. HRESIMS measurements were performed on a Shimadzu LCMS-IT-TOF liquid chromatograph mass spectrometer with ESI interface. TLC was performed on Kieselgel 60 F 254 (0.20 nm, Merck), and spots were viewed under ultraviolet light at 254 and 356 nm or stained by spraying with 50% H$_2$SO$_4$ and then heating. For column

chromatography, silica gel 60 (70−230 and 230−400 mesh, Merck) and Sephadex LH-20 were used. HPLC on compounds **1** and **2** used Shimadzu cat.# 2209139400; 30%−90%−100% MeCN, 0−10−16 min, gradient; flow rate 0.3 mL/min.

Plant Material. Plant material (5.42 kg) was collected in Pingtung County, Taiwan, in January 2006 and identified by one of the authors (M.-H.Y.). A voucher specimen (CARE200601) was deposited at the Graduate Institute of Natural Products, KMU, Kaohsiung, Taiwan.

Extraction and Isolation. Dry roots (5.42 kg) of *C. arisanensis* were extracted five times with MeOH (5 × 15 L) at room temperature to obtain a crude extract (224.9 g), which showed significant cytotoxic activity (EC_{50} < 20 μg/mL) against the KB cancer cell line. The crude extract was then partitioned with EtOAc−H_2O (1:1), and an EtOAc extract (32.7 g) was obtained. The EtOAc-soluble part showed inhibitory activity against A549, PC-3, DU145, HCT-8, MCF-7, KB, and KBVIN cell lines (IC_{50} < 20 μg/mL). Initial fractionation of the EtOAc extract was carried out by open column liquid chromatography on silica gel (4000 mL; 0.063−0.200 mm) using gradients of $CHCl_3$−MeOH and gave 13 fractions. Fractions 4−9 were subjected to additional chromatography as described below to obtain the new and known compounds.

Fraction 4 (3.24 g) was chromatographed on Sephadex LH-20 (diameter 5 cm, length 50 cm; CH_2Cl_2−MeOH, 1:1), and then subfraction 4-3 (957.30 mg) on Sephadex LH-20 (diameter 4 cm, length 40 cm; EtOAc−MeOH, 1:1) to yield two fractions. The second fraction (159.5 mg) was purified on a silica gel column (425 mL; 40−63 μm), eluted with CH_2Cl_2, to give **1** (6.0 mg), **7** (7.30 mg), and **8** (12.6 mg).

Fraction 5 (1.05 g) was separated into four fractions on Sephadex LH-20 (diameter 4 cm, length 40 cm) using CH_2Cl_2−MeOH (1:1) as the eluent. Subfraction 5-2 (269.4 g) was chromatographed on a silica gel column (510 mL; 40−63 μm) using CH_2Cl_2 as the eluent, and subfraction 5-2-2 (126.40 mg) was subsequently purified with *n*-hexane−EtOAc (initially 2:1, with increasing ratio of EtOAc) to obtain **2** (7.7 mg) and **6** (5.0 mg).

Fraction 6 (656.4 mg) was chromatographed over Sephadex LH-20 (diameter 4 cm, length 50 cm; CH_2Cl_2−MeOH, 1:1), and then subfraction 6-2 (334.3 mg) was subjected to Sephadex LH-20 (diameter 4 cm, length 40 cm; EtOAc−MeOH, 1:1). Subfraction 6-2-2 was purified on a silica gel column (310 mL; 40−63 μm) using *n*-hexane−EtOAc (3:1) as the eluent to give **9** (22.4 mg).

Fraction 7 (424.0 mg) was chromatographed on Sephadex LH-20 (diameter 5 cm, length 50 cm; CH_2Cl_2−MeOH, 1:1) to give three subfractions. The third subfraction was purified by column chromatography over silica gel eluting with *n*-hexane−EtOAc (3:1) to yield **3** (13.8 mg), **5** (21.3 mg), and **4** (8.9 mg).

Fraction 8 (1.25 g) was chromatographed using Sephadex LH-20 (diameter 5 cm, length 50 cm; CH_2Cl_2−MeOH, 1:1). Subfraction 8-3 (347.9 mg) was then subjected to Sephadex LH-20 (diameter 4 cm, length 40 cm; EtOAc−MeOH, 1:1) chromatography, which yielded four fractions. The third fraction (52.2 mg) was chromatographed on silica gel (350 mL; 40−63 μm) eluted with *n*-hexane−EtOAc (3:1) to give **10** (10.7 mg).

Fraction 9 (1.7 g) was separated into three subfractions by chromatography on Sephadex LH-20 using CH_2Cl_2−MeOH (1:1). The third subfraction was rechromatographed over silica gel eluting with *n*-hexane−EtOAc (1:1) to yield **11** (9.3 mg).

Calanquinone B (2): brown, amorphous solid; mp 192−193 °C; UV (MeOH) λ_{max} (log ε) 249 (4.39), 296 (4.11), 406 (3.39) nm; IR (neat) ν_{max} 3380, 2926, 2852, 1682, 1640, 1616, 1478, 1427, 1289, 1233, 1198, 1167, 1111, 1086, 1006, 842 cm⁻¹; ¹H and ¹³C NMR ($CDCl_3$), see Tables 1 and 2; HRESIMS *m/z* 313.0690 (calcd for $C_{17}H_{14}O_6$ − H, 313.0712).

Calanquinone C (3): red, amorphous solid; mp 107−108 °C; UV (MeOH) λ_{max} (log ε) 218 (4.13), 273 (3.77), 322 (3.52) nm; IR (neat) ν_{max} 3386, 2935, 2853, 1646, 1607, 1560, 1466, 1385, 1357, 1341, 1284, 1238, 1192, 1094, 915, 835 cm⁻¹; ¹H and ¹³C NMR (C_5D_5N), see Tables 1 and 2; HRESIMS *m/z* 287.0922 (calcd for $C_{16}H_{14}O_5$ + H, 287.0920).

Calanhydroquinone A (4): red, amorphous solid; mp 97−98 °C; UV (MeOH) λ_{max} (log ε) 216 (4.22), 273 (3.84), 308 (3.74) nm; IR (neat) ν_{max} 3261, 2922, 2850, 1613, 1561, 1462, 1283, 1264, 1234,

1192, 1096, 801 cm⁻¹; ¹H and ¹³C NMR (C_5D_5N), see Tables 1 and 2; HRESIMS *m/z* 287.0896 (calcd for $C_{16}H_{16}O_5$ − H, 287.0920).

Calanhydroquinone B (5): red, amorphous solid; mp 135−136 °C; UV (MeOH) λ_{max} (log ε) 217 (4.38), 269 (4.01), 312 (3.79), 369 (3.34) nm; IR (neat) ν_{max} 3288, 2940, 2841, 1605, 1572, 1460, 1450, 1429, 1332, 1283, 1233, 1193, 1171, 1077, 1012, 964, 929, 815 cm⁻¹; ¹H and ¹³C NMR (C_5D_5N), see Tables 1 and 2; HRESIMS *m/z* 287.0902 (calcd for $C_{16}H_{16}O_5$ − H, 287.0920).

Calanhydroquinone C (6): red, amorphous solid; mp 97−98 °C; UV (MeOH) λ_{max} (log ε) 216 (4.27), 269 (3.85), 321 (3.54), 367 (3.23) nm; IR (neat) ν_{max} 3476, 3280, 2943, 2849, 1712, 1662, 1592, 1558, 1457, 1407, 1357, 1274, 1234, 1195, 1119, 1102, 1056, 966 cm⁻¹; ¹H and ¹³C NMR (C_5D_5N), see Tables 1 and 2; HRESIMS *m/z* 301.1051 (calcd for $C_{17}H_{18}O_5$ − H, 301.1076).

Calanphenanthrene A (7): brown, amorphous solid; mp 163−164 °C; UV (MeOH) λ_{max} (log ε) 219 (4.40), 274 (4.03), 307 (3.87) nm; IR (neat) ν_{max} 3409, 3227, 2941, 2837, 1615, 1480, 1450, 1428, 1352, 1277, 1199, 1153, 1094, 1052, 1000, 981, 953, 924, 844 cm⁻¹; ¹H and ¹³C NMR (C_5D_5N), see Tables 1 and 2; HRESIMS *m/z* 271.0984 (calcd for $C_{16}H_{16}O_4$ − H, 271.0970).

In Vitro Cytotoxicity Bioassays.[8] All stock cultures were grown in T-25 flasks. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 5000 cells per well with compounds added from DMSO-diluted stock. After three days in culture, attached cells were fixed with cold 10% trichloroacetic acid and then stained with 0.4% sulforhodamine B. The absorbency at 562 nm was measured using a microplate reader after solubilizing the bound dye. The mean EC_{50} is the concentration of agent that reduces cell growth by 50% under the experimental conditions and is the average from at least three independent determinations that were reproducible and statistically significant. The following human tumor cell lines were used in the assay: human lung (A549), prostate (PC-3 and DU145), colon (HCT-8), breast (MCF-7), nasopharyngeal (KB), and vincristine-resistant nasopharyngeal (KBVIN) cancer cell lines. All cell lines were obtained from the Lineberger Cancer Center (UNC-CH) or from ATCC (Rockville, MD) and cultured in RPMI-1640 medium supplemented with 25 mM HEPES, 0.25% sodium bicarbonate, 10% fetal bovine serum, and 100 μg/mL kanamycin.

Supporting Information Available: The ¹H, ¹³C, HSQC, HMBC, and NOESY spectra of compounds **1**−**7** are available free of charge via the Internet at http://pubs.acs.org.

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