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Cytotoxic Calanquinone A from *Calanthe arisanensis* and Its First Total Synthesis

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Abstract

Calanquinone A (**1**) was isolated from an EtOAc-soluble extract of *Calanthe arisanensis* through bioassay-guided fractionation. Its structure was identified by spectroscopic methods. Compound **1** showed potent cytotoxicity (EC_{50} < 0.5 μg/mL) against lung (A549), prostate (PC-3 and DU145), colon (HCT-8), breast (MCF7), nasopharyngeal (KB), and vincristine-resistant nasopharyngeal (KB-VIN) cancer cell lines, and interestingly, showed an improved drug resistance profile compared to paclitaxel. The total synthesis of **1** was also achieved and reported herein.

The *Calanthe* genus 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 nineteen are found in Taiwan. Among them *Calanthe arisanensis* Hayata is endemic to Taiwan and grows in forests from 1000 to 2000 m throughout the island.[1]

A phytochemical study of this plant has not been reported to date. In cytotoxicity screening of extracts of Formosan plants, an EtOAc extract of *C. arisanensis* was found to be active against various human cancer cell lines with IC_{50}< 20 μg/mL. Bioassay-directed chromatographic fractionation of this extract produced a new phenanthraquinone calanquinone A (**1**). Compound **1** showed significant in vitro cytotoxic activicity against seven human cancer cell lines, as described below.

The active MeOH extract (225 g) of dry roots of *C. arisanensis* (5.42 kg) was partitioned between EtOAc and water (1:1, v/v). Further fractionation of the active EtOAc extract (32.7 g) by repeated liquid chromatography on silica gel gave calanquinone A (**1**). HRESIMS of **1**

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showed a $[M-H]^-$ ion at *m/z* 313.0705 ($C_{17}H_{14}O_6$ -H), indicating 11 degrees of unsaturation. The IR spectrum showed absorptions for hydroxyl (3348 cm^{-1}), carbonyl (1642 cm^{-1}), and aromatic ring (1626, 1514, 1460, 1411, and 844 cm^{-1}) functional groups. UV absorptions at 242, 308, and 426 nm also indicated an aromatic system. Seventeen carbon signals, including three methoxy, four methine, and ten quaternary carbons, were observed in the NMR spectra of **1** (Table 1). Among the ten quaternary carbons, two were identified as carbonyl carbons on the basis of chemical shifts δ_C 184.7 and 186.2. Therefore, the data supported the presence of two carbonyls, six olefins, and three ring moieties to fulfill the 11 degrees of unsaturation, and **1** was postulated to be an phenanthrenedione or anthrenedione.[2] 1D NMR and HSQC data indicated the presence of three methoxy groups at δ_H 3.96, 4.01, and 4.02 (δ_C 57.1, 56.2, and 61.0), one pair of *ortho*-coupled aromatic protons at δ_H 8.05 (d, *J*=8.7 Hz, δ_C 137.1) and 8.10 (d, *J*=8.7 Hz, δ_C 122.0), and two olefinic protons at δ_H 6.15 (s, δ_C 107.4) and 6.86 (s, δ_C 101.4). In the HMBC spectra, the olefinic proton at δ_H 6.86 exhibited ^2J interactions with a carbon at δ_C 155.2 (C-7), as well as ^3J interactions with carbons at δ_C 118.7 (C-4b), 140.4 (C-6), and 137.1 (C-9). The other olefinic proton at δ_H 6.15 exhibited ^2J interactions with a carbon at δ_C 161.7 (C-3), as well as ^3J interactions with carbons at δ_C 186.2 (C-4) and 133.0 (C-10a). Locations of methoxy groups at C-3, C-6, and C-7 were confirmed by the following NOESY correlations: δ_H 6.15 (H-2)/3.96 (3-OMe), δ_H 6.86 (H-8)/4.01 (7-OMe) and 8.05 (H-9), δ_H 4.01 (7-OMe)/4.02 (6-OMe), and δ_H 8.05 (H-9)/8.10 (H-10). Thus, compound **1** was identified as 5-hydroxy-3,6,7-trimethoxy-1,4-phenanthrenequinone and has been named as calanquinone A (**1**).

Compound **1** is related in structure to other naturally occurring phenanthrenequinones, including the des-oxy analog sphenone (lacking the C-5 OH group),[3] cymbinodin A (lacking the two methoxy groups at C-6 and C-7),[4] and annoquinone A (lacking any substituents on ring C).[5,6] In prior studies, sphenone and annoquinone A showed cytotoxic activity against the KB cell line (reported EC_{50} 2.7[3] and 1.6[5] μg/mL, respectively).

Compound **1** exhibited potent cytotoxicity (EC_{50} 0.03–0.45 μg/mL) against human lung (A549), prostate (PC-3 and DU145), colon (HCT-8), breast (MCF7), nasopharyngeal (KB), and vincristine-resistant nasopharyngeal (KB-VIN) cancer cell lines. Paclitaxel was used as a positive control (data shown in Table 2). Interestingly, **1** exhibited comparble potency against both KB and its drug-resistant KB-VIN subline, and thus, showed an improved drug resistance profile compared to paclitaxel. The cytotoxic values demonstrate the strong potential of **1** as a promising lead compound and *C. arisanensis* as a promising plant source of new agents for cancer chemotherapy.

In order to make sufficient quantities of **1** for extensive biological evaluation, we modified the synthetic procedure of Kraus and co-workers[7,8] (Scheme 1) to synthesize **1**. As shown in Scheme 2, we prepared 2-methoxy-5-carboxylic acid methyl ester-1,4-quinone (**4**)[9] by AgO oxidation of the methyl ester (**3**) of commercially available 2,4,5-trimethoxybenzoic acid (**2**). Compound **4** was coupled with 3,4,5-trimethoxytoluene in the presence of 1 equiv. of trifluoroacetic acid to produce quinone **5**, although Kraus reported the production of hydroquinone **10** under these reaction conditions (Scheme 1). The quinone skeleton of **5** was confirmed from ^{13}C-NMR data, which showed two carbonyl groups at 180.6 and 183.6 ppm. In addition, reaction of **5** with Me_2SO_4 did not give a methoxylated product (i.e., **11** in Scheme 1). We attempted to reduce **5** with $Na_2S_2O_4$ to obtain the corresponding hydroquinone, which could be transformed to the Kraus type of intermediate **11** (R = OMe) after methylation. Despite extending the reaction time and adding more reducing agent, only starting material was recovered.

Therefore, **5** was reduced with LAH (THF, reflux, 1 h) to alcohol, which was oxidized selectively to aldehyde **6** with activated MnO_2 (toluene, 110 °C, overnight) (Scheme 2). After

an unsuccessful attempt to obtain the phenanthraquinone **9** directly from **6** using P_4-*t*Bu, quinone **6** was first reduced to the hydroquinone using aq. $Na_2S_2O_4$ (CH_2Cl_2, rt., overnight),[10] and then methylated with Me_2SO_4 in the presence of K_2CO_3(acetone, 60 °C, 1.5 h) to give the desired compound **7** (Scheme 2). Cyclization of **7** with P_4-*t* Bu (benzene, 100 °C, 63 h) gave **8**, which was oxidized with AgO (6N HNO_3, acetone, 50 °C, 2–3 min) to phenanthraquinone **9**. Compound **9** was converted to calanquinone A (**1**) by selective demethylation with TMSI in CH_2Cl_2 at r.t. (Scheme 2).

Synthesized **1** and intermediates **5–9** were screened in an *in vitro* cytotoxicity assay (data shown in Table 3). Compound **1** exhibited the highest potency (EC_{50} 0.15–0.75 μg/mL) against all seven tested cancer cell lines. The remaining compounds showed no (**6–8**) or only weak (**5** and **9**) activity. Clearly, the potency of **1** merits further study and our synthetic route can efficiently produce sufficient quantities of **1** for future extensive biological evaluation and SAR investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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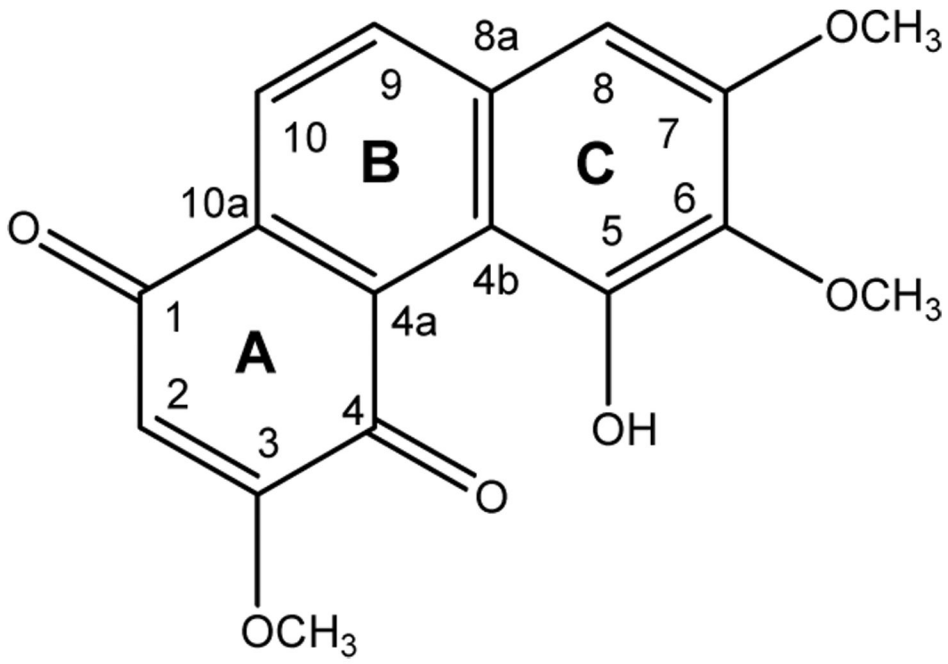
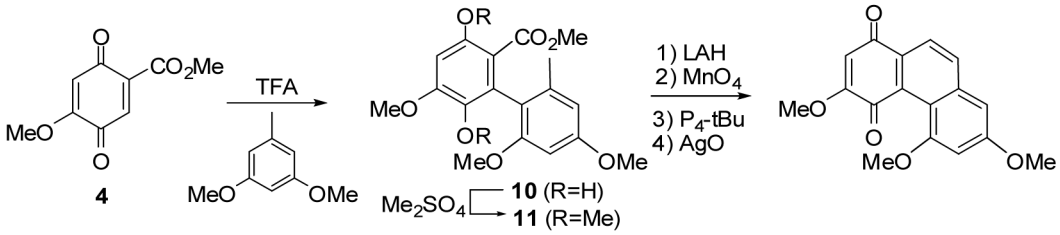
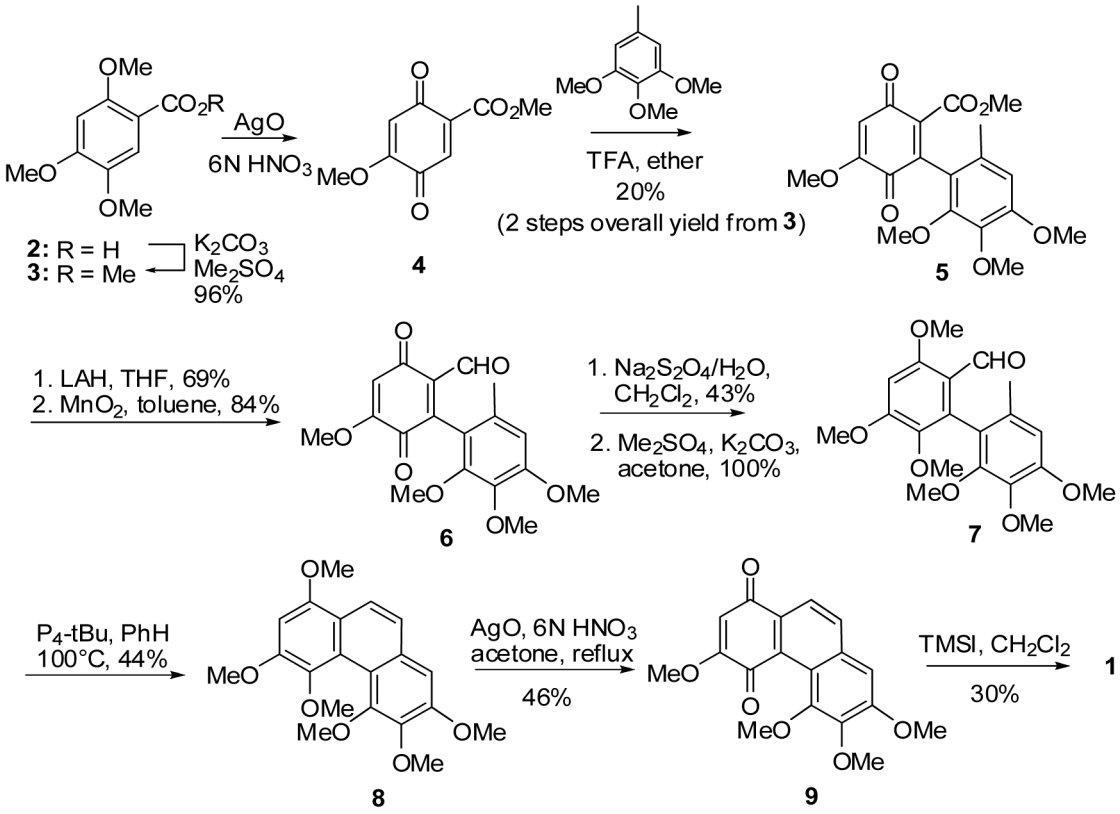


Figure 1.
Structure of calanquinone A (**1**).



Scheme 1.
Synthetic procedure of Kraus.



Scheme 2.
Our total synthesis of calanquinone A (**1**).

Table 1

NMR data of calanquinone A (**1**)[a]

Proton	^{13}C (δ_C)	^{1}H (δ_H)	HMBC (^{13}C no)	NOESY (^{1}H no)
1	184.7			
2	107.4	6.15 s	3, 4, 10a	C_3-OC<u>H</u>$_3$
3	161.7			
4	186.2			
4a	128.3			
4b	118.7			
5	148.3			
6	140.4			
7	155.2			
8	101.4	6.86 s	4b, 6, 7, 9	9, C_7-OC<u>H</u>$_3$
8a	135.1			
9	137.1	8.05 d (8.7)	4b, 8, 10a	8, 10
10	122.0	8.10 d (8.7)	1, 4a, 8a	9
10a	133.0			
−OCH_3	C_3-O<u>C</u>H$_3$ 57.1	C_3-O<u>C</u>H$_3$ 3.96 s	3	2
	C_6-O<u>C</u>H$_3$ 61.0	C_6-O<u>C</u>H$_3$ 4.02 s	6	C_7-OC<u>H</u>$_3$
	C_7-O<u>C</u>H$_3$ 56.2	C_7-O<u>C</u>H$_3$ 4.02 s	7	8, C_6-OC<u>H</u>$_3$
C_5-OH		10.73		

[a]Measured in $CDCl_3$ (300 and 500 MHz, δ in ppm, *J* in Hz).

Table 2

Cytotoxicity of calanquinone A (**1**) isolated from *C. arisanensis*

	EC_{50} (μg/mL) / Cell line						
Compound	**A549**	**PC-3**	**DU145**	**HCT-8**	**MCF-7**	**KB**	**KB-VIN**
Calanquinone A (**1**)	0.19	0.16	0.34	0.20	0.03	0.32	0.45
Paclitaxel[a]	<0.005	0.0097	<0.005	0.21	0.0072	<0.005	2.16

[a] positive control

Table 3

Cytotoxicity of synthesized **1** and related intermediates

	EC_{50} (μg/mL) / Cell line						
Compound	**A549**	**PC3**	**DU145**	**HCT8**	**MCF7**	**KB**	**KB-VIN**
1	0.31	0.75	0.48	0.29	0.15	0.30	0.24
5	6.12	6.09	4.74	5.85	6.40	4.02	5.48
6	NA	NA	NA	NA	NA	NA	NA
7	NA	NA	NA	NA	NA	NA	NA
8	NA	NA	NA	NA	NA	NA	NA
9	7.06	7.81	4.40	15.25	5.33	8.08	9.14

NA: no activity up to 20 μg/mL