Bioorganic & Medicinal Chemistry Letters 18 (2008) 4275–4277

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Cytotoxic calanquinone A from *Calanthe arisanensis* and its first total synthesis

Chia-Lin Lee [a,b], Kyoko Nakagawa-Goto [a], Donglei Yu [a], Yi-Nan Liu [a], Kenneth F. Bastow [c], Susan L. Morris-Natschke [a], Fang-Rong Chang [b], Yang-Chang Wu [b,d,*], Kuo-Hsiung Lee [a,*]

[a] Natural Products Research Laboratories, School of Pharmacy, University of North Carolina at Chapel Hill, 315 Beard Hall, CB# 7360, NC 27599-7360, USA
[b] Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan
[c] Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA
[d] Center of Excellence for Environmental Medicine, Kaohsiung Medical University, Kaohsiung 807, Taiwan

ARTICLE INFO

Article history:
Received 27 March 2008
Revised 26 June 2008
Accepted 30 June 2008
Available online 3 July 2008

Keywords:
Calanquinone A
Calanthe arisanensis
Cytotoxicity
Total synthesis

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 is 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 activity against seven human cancer cell lines, as described below (see Fig. 1).

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** 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

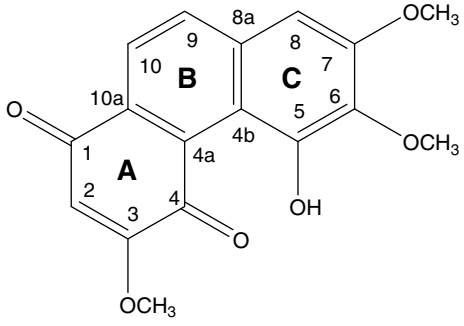


Figure 1. Structure of calanquinone A (**1**).

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 a 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

* Corresponding authors. Tel.: +886 7 312 1101x2197; fax: +886 7 311 4773 (Y.C.W.); Tel.: +1 919 962 0066; fax: +1 919 966 3893 (K.H.L.).
E-mail addresses: yachwu@kmu.edu.tw (Y.-C. Wu), khlee@unc.edu (K.-H. Lee).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.06.099

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–OCH_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–OCH_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–$OCH_3$57.1	C_3–$OCH_3$3.96 s	3	2
	C_6–$OCH_3$61.0	C_6–$OCH_3$4.02 s	6	C_7–OCH_3
	C_7–$OCH_3$56.2	C_7–$OCH_3$4.02 s	7	8, C_6–OCH_3
C_5–OH		10.73		

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

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 comparable 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 (6 N 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 rt (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** mer-

Table 2
Cytotoxicity of calanquinone A (**1**) isolated from *C. arisanensis*

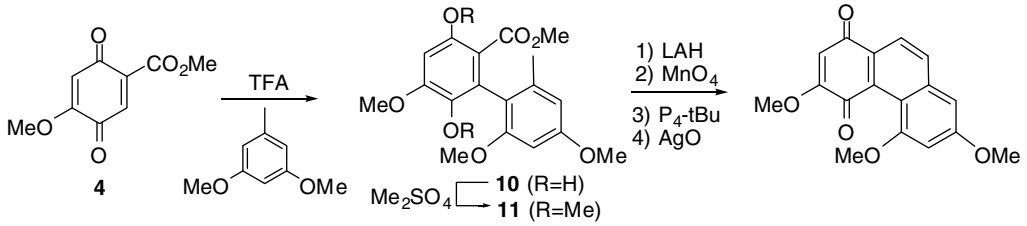
Compound	EC_{50} (μg/mL)/cell line						
	A549	PC-3	DU145	HCT-8	MCF-7	KB	KBVIN
Calanquinone A (**1**)	0.19	0.16	0.34	0.20	0.03	0.32	0.45
Paclitaxel[a]	<0.005	0.0097	<0.005	0.21	0.0072	<0.005	2.16

[a] Positive control.

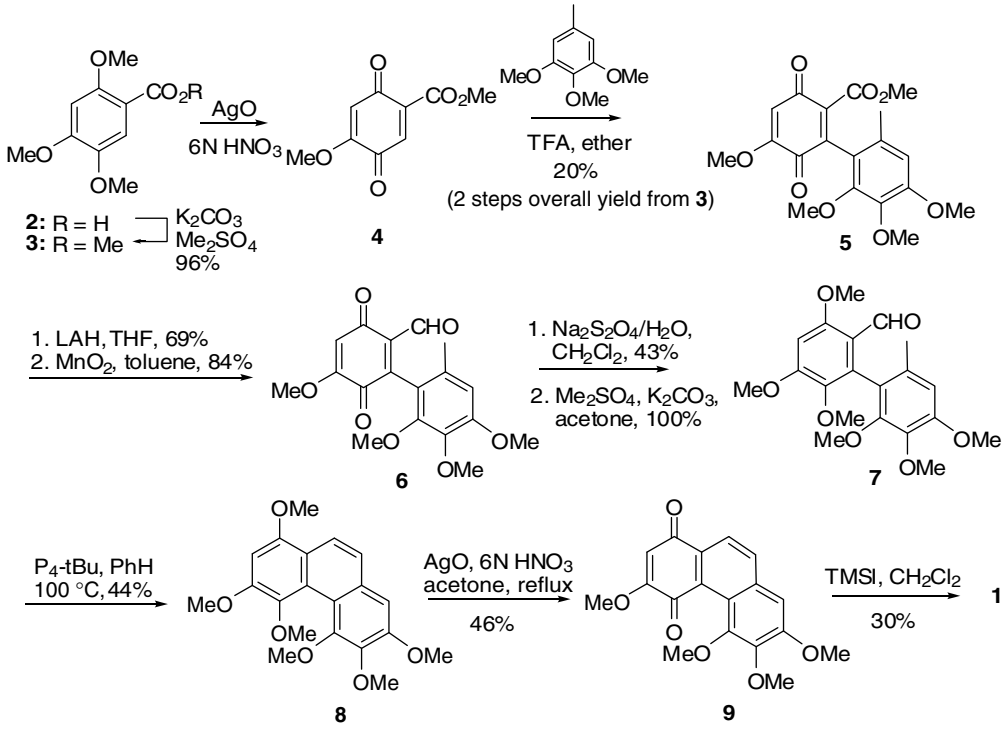
Table 3
Cytotoxicity of synthesized **1** and related intermediates

Compound	EC_{50} (μg/mL)/cell line						
	A549	PC3	DU145	HCT8	MCF7	KB	KBVIN
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.



Scheme 1. Synthetic procedure of Kraus.



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

its further study and our synthetic route can efficiently produce sufficient quantities of **1** for future extensive biological evaluation and SAR investigation.

Acknowledgments

This work was supported by grants from National Cancer Institute, NIH, USA (CA 17625, K.H. Lee), National Science Council, Taiwan (Y.-C. Wu) and KMU-EM-97-2.1.b (Y.-C. Wu).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.06.099.

References and notes

1. Boufford, D. E.; Hsieh, C.-F.; Huang, T.-C.; Kuoh, C.-S.; Ohashi, H.; Su, H.-J.; Yeh, H.-Y.; Hsiao, J.-L.; Hsiao, S.-H. *Flora of Taiwan*; Editorial Committee of the Flora of Taiwan: Taipei, Taiwan, **2000**; Vol. 5, pp. 776–777, 782–783.
2. Fan, C.; Wang, W.; Wang, Y.; Qin, G.; Zhao, W. *Phytochemistry* **2001**, *57*, 1255.
3. Wu, T. S.; Huang, S. C.; Chen, M. T.; Jong, T. T. *Phytochemistry* **1989**, *28*, 1280.
4. Barula, A. K.; Ghosh, B. B.; Ray, S.; Patra, A. *Phytochemistry* **1990**, *29*, 3056.
5. Wu, T. S.; Jong, T. T.; Tien, H. J.; Kuoh, C. S.; Furukawa, H.; Lee, K. H. *Phytochemistry* **1987**, *26*, 1623.
6. Krohn, K.; Loock, U.; Paavilainen, K.; Hausen, B.; Schmalle, H. W.; Kiesele, H. *ARKIVOC* **2001**, *2*, 88.
7. Kraus, G. A.; Hoover, K.; Zhang, N. *Tetrahedron Lett.* **2002**, *43*, 5319.
8. Kraus, G. A.; Zhang, N. . *Tetrahedron Lett.* **2002**, *43*, 9597.
9. Parker, K. A.; Spero, D. M.; Koziski, K. A. *J. Org. Chem.* **1987**, *52*, 183.
10. Hormi, O. E. O.; Moilanen, A. M. . *Tetrahedron* **1998**, *54*, 1943.