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Bi-bicyclic and bi-tricyclic compounds from *Dendrobium thyrsiflorum*

Guang-Nong Zhang [a], Ling-Yan Zhong [c], S.W. Annie Bligh [d], Ying-Li Guo [c], Chao-Feng Zhang [a], Mian Zhang [a], Zheng-Tao Wang [a,b,*], Luo-Shan Xu [a]

[a] *Department of Pharmacognosy, China Pharmaceutical University, Nanjing 210038, China*
[b] *Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Zhangjiang Hi-Tech Park, Shanghai 201203, China*
[c] *Shanghai Genomics Inc., 640 Song Tao Road, Zhangjiang Hi-Tech Park, Shanghai 201203, China*
[d] *Institute for Health Research and Policy, London Metropolitan University, 166-220 Holloway Road, London N7 8DB, UK*

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Abstract

One bi-bicyclic and two bi-tricyclic derivatives of coumarin–benzofuran, phenanthrene–phenanthrene and phenanthrene–phenanthraquinone, along with seven known compounds, were isolated from stems of *Dendrobium thyrsiflorum* Rchb.f. (Orchidaceae). On the basis of chemical, NMR (^{1}H, ^{13}C, HMQC, HMBC and NOESY) and mass spectrometry data, their structures were elucidated as denthyrsin [3-(5′,6′-dimethoxybenzofuran-2′-yl)-6,7-dimethoxy-2H-chromen-2-one; **1**], denthyrsinol (4,5′-dimethoxy-[1,1′]biphenanthrenyl-2,5,4′,7′-tetraol; **2**), and denthyrsinone (7,4′,7′-trihydroxy-2,2′,8′-trimethoxy-[5,1′]biphenanthrenyl-1,4-dione; **3**). Compounds **1**–**3** and denthyrsinin (1,5,7-trimethoxyphenanthrene-2,6-diol; **4**) showed significant cytotoxic activities against Hela (13.5, 9.3, 9.9 and 2.7 μM, respectively), K-562 (0.45, 1.6, 6.0 and 2.3 μM, respectively) and MCF-7 (18.1, not tested, 3.5 and 4.8 μM, respectively) cell lines.

Keywords: *Dendrobium thyrsiflorum*; Orchidaceae; Denthyrsin; Denthyrsinol; Denthyrsinone; Cytotoxic activity

1. Introduction

The genus *Dendrobium* is represented by more than 1100 species widely distributed throughout Asia, Europe and Australia, thus being the richest genus of the family Orchidaceae. There are 74 species and 2 variations of *Dendrobium* plants found in China and about 30 species of them are used in traditional or folk medicine in China as a Yin tonic to nourish the stomach, promote the production of body fluid and reduce fever (Zhang et al., 2003). Some of the species are cultivated for ornamental purposes. Recent pharmacological studies have shown that some species displayed antitumor (Lee et al., 1995; Morita et al., 2000; Ma et al., 1994), anti-angiogenic (Gong et al., 2004), anti-platelet aggregation (Fan et al., 2001), anti-inflammation (Lin et al., 2001) and immunoregulatory activities (Zhao et al., 2001).

The chemical constituents of this genus have been extensively investigated. Zhang et al. (2003) reviewed 100 compounds isolated from 42 *Dendrobium* species, including 32 alkaloids, 6 coumarins, 15 bibenzyls, 4 fluorenones, 22 phenanthrenes and 7 sesquiterpenoids. Recently the isolation and identification of five new sesquiterpenes (Zhao et al., 2003a) and four sesquiterpene glycosides (Zhao et al., 2003b), two new fluorenones (Yang et al., 2004), lignans (Ye et al., 2004), as well as a heteropolysaccharide (Hua et al., 2004), were reported.

* Corresponding author. Tel.: +86 21 51322507; fax: +86 21 51322519.
E-mail address: wangzht@shutcm.com (Z.-T. Wang).

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Dendrobium thyrsiflorum Rchb.f. is not recorded in the China Pharmacopoeia (2000 edition); however, it has been commonly used as a substitute for Herba Dendrobii due to its wide distribution and similar morphologies to the officially recorded species. In this species only three coumarins were reported (Wrigley, 1960). In the continuation of pharmacognostical and chemical investigation into *Dendrobium* plants, the ethanol extract of the stem of this species was examined and 23 compounds were isolated. Thirteen known compounds, including moscatin (**6**) and hircinol (**7**), were identified by Zhang et al. (2004). In this paper, the isolation and identification of three new and seven known compounds, as well as the cytotoxic activities of some selected compounds, are reported. On the basis of chemical, NMR (^{1}H, ^{13}C, HMQC, HMBC and NOESY) and mass spectrometry data, the structures of the new compounds were elucidated as denthyrsin [3-(5′,6′-dimethoxybenzofuran-2′-yl)-6,7-dimethoxy-2H-chromen-2-one; **1**], denthyrsinol (4,5′-dimethoxy-[1,1′]biphenanthrenyl-2,5,4′,7′-tetraol; **2**), and denthyrsinone (7,4′,7′-trihydroxy-2,2′,8′-trimethoxy-[5,1′]biphenanthrenyl-1,4-dione; **3**). (Fig. 1). The known compounds were characterized as denthyrsinin (1,5,7-trimethoxyphenanthrene-2,6-diol, **4**; Majumder and Sen, 1991), scoparone (**5**; Sankar et al., 1982), chrysophanol (Danielsen and Aksnes, 1992), physcion (Danielsen and Aksnes, 1992), emodin (Danielsen and Aksnes, 1992), β-sitosterol (Kojima et al., 1990), and daucosterol (Kojima et al., 1990).

The cytotoxicities of seven selected compounds from *D. thyrsiflorum* against several tumor cell lines were determined by MTT assay. Compounds **1**–**4** showed significant cytotoxicity to Hela, K-562 and MCF-7 cell lines.

2. Results and discussion

The air-dried stems of *D. thyrsiflorum* Rchb.f. were extracted with EtOH and the filtrate was evaporated to dryness. The residue was suspended in water and partitioned with EtOAc and then *n*-BuOH to give three fractions (EtOAc phase, *n*-BuOH phase and aqueous phase). The EtOAc and *n*-BuOH extracts were individually subjected to repeated silica gel, RP-18 and Sephadex LH-20 chromatographic purification to afford 10 compounds.

Compound **1** was obtained as yellow needles, m.p. 262.1–263.0 °C. Its molecular formula was established as $C_{21}H_{18}O_7$ by HREIMS giving a molecular ion at *m*/*z* 382.1048. The UV spectrum exhibited maxima at 263, 286, 318 and 337 nm which were similar to coumarin derivatives (Ke and Dong, 1998). The IR spectrum showed strong bands for carbonyl (1717 cm^{-1}) and aro-

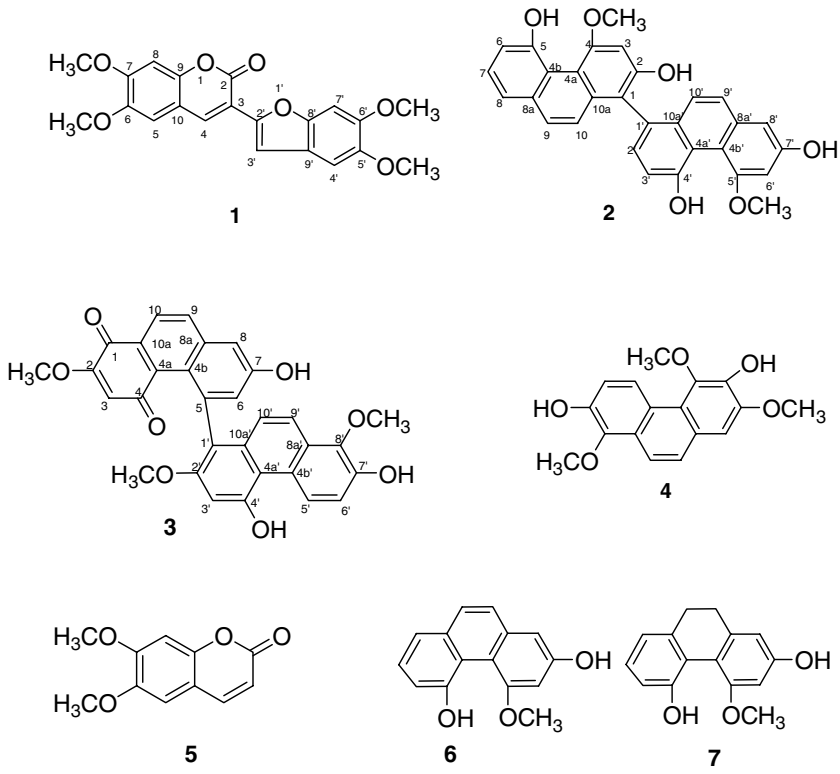

Fig. 1. Structures of compounds **1**–**7**.

matic ring groups (3086, 1618, 975 and 618 cm^{-1}). The ^1H NMR spectrum of **1** exhibited six singlets assigned to aromatic protons at δ6.88–8.21 and four methoxyl singlets at δ3.93–3.97. The ^{13}C NMR (Table 1) and HMQC spectra revealed **1** contains eleven quaternary carbons, six methine carbons and four methoxyl carbons. Among the eleven quaternary carbons, the signal at δ158.6 was typical of the carbonyl carbon of coumarin derivatives (Sankar et al., 1982), and the four downfield signals at δ153.0, 146.9, 149.7 and 147. 1 were attributed to the four methoxyl-substituted aromatic carbons. On the basis of its ^1H and ^{13}C NMR spectroscopic data, the skeleton of **1** was identified as a coumarin–benzofuran.

In the HMBC spectrum of **1** (Table 1), correlations were found between H-8 (δ6.88) and C-7 (δ146.9) and C-9 (δ148.9), and between H-5 (δ6.98) and C-6 (δ153.0) and C-10 (δ112.2), indicating that the coumarin was substituted at C-6 and C-7. The singlet at δ8.21 (H-4) and the absence of H-3 indicated that C-3 of the coumarin was the linkage site (Sankar et al., 1982).

For the benzofuran residue, HMBC correlations were found between H-7′ (δ7.04) and C-6′ (δ147.1) and C-8′ (δ149.7), and between H-4′ (δ7.04) and C-5′ (δ149.7) and C-9′ (δ121.5), giving a 5′,6′-substituted benzofuran moiety. The singlet at δ7.62 (H-3′) and the absence of H-2′ indicated that the coumarin and benzofuran moieties were connected through C-3 and C-2′ (Yu and Yang, 1999).

The C-2′ and C-3 coupling was further confirmed by the cross peaks between H-4 and C-2′, and between H-3′ and C-3 in the HMBC spectrum. Thus, the structure of **1** was established to be 3-(5′,6′-dimethoxybenzofuran-2′-yl)-6,7-dimethoxy-2H-chromen-2-one, named denthyrsin, which is a new coumarin–benzofuran derivative.

Table 1
^1H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data of denthyrsin (**1**) in $CDCl_3$

Denthyrsin (**1**)			
Carbon	^1H, δ	^{13}C, δ	HMBC[a]
2		158.6	
3		115.4	
4	8.21 *s*	134.8	2, 3, 5, 10, 2′
5	6.98 *s*	108.3	4, 6, 7, 9, 10
6		153.0	
7		146.9	
8	6.88 *s*	99.8	6, 7, 9, 10
9		148.9	
10		112.2	
2′		149.1	
3′	7.62 *s*	108.4	3, 2′, 4′, 8′, 9′
4′	7.04 *s*	103.2	3′, 5′, 6′, 9′
5′		149.7	
6′		147.1	
7′	7.04 *s*	95.1	5′, 6′, 8′, 9′
8′		149.7	
9′		121.5	
6-OMe	3.97	56.4	6
7-OMe	3.93	56.4	7
5′-OMe	3.95	56.4	5′
6′-OMe	3.96	56.4	6′

[a] Carbons that correlate with the proton resonance.

Compound **2** was obtained as a white amorphous powder, m.p. 361.3–362.9 °C. HREIMS of **2** exhibited a molecular ion peak at *m/z* 478.1424 $[M]^+$. The appearance of an intense doubly charged molecular ion at *m/z* 239 (half of the molecular ion, *m/z* 478) in the mass spectrum indicated that **2** was built up of two monomeric moieties of equal elemental composition ($C_{15}H_{11}O_3$). The UV spectrum of **2** showed absorption maxima at 214, 255, 286 and 321 nm which were similar to those of phenanthrene derivatives (Majumder and Sen, 1987). The phenolic nature of compound **2** was indicated by its characteristic color reaction with ferric iron and its IR spectrum exhibiting absorption at 3401 cm^{-1} (OH) and 1617, 1589, 1533, 827 and 659 cm^{-1} (aromatic rings).

The ^1H and ^{13}C NMR spectra of **2** (Table 2) implied an asymmetrical dimeric phenanthrene derivative by comparison with analogous compounds (Majumder and Lahiri, 1990; Majumder et al., 1998, 1999). The four proton signals at δ9.46, 9.36, 9.04 and 8.08 in the ^1H NMR spectrum disappeared on deuterium exchange,

Table 2
^1H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data of denthyrsinol (**2**) in acetone-d_6

Denthyrsinol (**2**)			
Carbon	^1H, δ (*J*, Hz)	^{13}C, δ	HMBC[a]
1		119.1	
2		155.9	
3	7.19 *s*	102.8	1, 2, 4, 4a
4		156.4	
5		155.8	
6	7.13 (*dd*, 1.5, 7.7)	117.7	5, 8, 4b
7	7.43 (*t*, 7.7)	130.1	5, 8, 8a
8	7.36 (*dd*, 1.5, 7.7)	121.7	6, 7, 4b
9	7.15 (*d*, 9.1)	127.8	8, 4b, 10a
10	6.95 (*d*, 9.1)	125.6	1, 4a, 8a
4a		114.9	
4b		120.6	
8a		135.3	
10a		137.6	
1′		121.3	
2′	7.44 (*d*, 7.7)	131.9	1, 4′, 1′, 10a′
3′	7.26 (*d*, 7.7)	117.6	4′, 1′, 4a′
4′		155.6	
5′		157.2	
6′	7.02 (*d*, 2.5)	103.2	5′, 8′
7′		158.3	
8′	7.01 (*d*,2.5)	108.2	6′, 7′, 4b′
9′	7.34 (*d*, 8.0)	127.9	4b′, 10a′
10′	7.39 (*d*, 8.0)	128.2	1′, 9′, 8a′, 10a′
4a′		126.9	
4b′		114.7	
8a′		135.0	
10a′		137.1	
OMe	4.22	59.2 (C-4)	
	4.24	59.3 (C-5′)	

[a] Carbons that correlate with the proton resonance.

indicated the presence of four phenolic hydroxyl groups in the compound, while the two three-proton singlets at δ4.24 and 4.21 corresponded to two aromatic methoxyls in different environments. In addition, the absence of any downfield aromatic protons below δ8.0 that could be attributed to H-4 /H-4′ or H-5/H-5′ protons of phenanthrene derivatives implied that both C-4/C-4′ and C-5 / C-5′ are substituted (Majumder and Sen, 1987). The ^{1}H NMR spectrum exhibited signals for three adjacent aromatic protons at δ7.13 (*dd*, J = 1.5, 7.7 Hz), 7.43 (*t*, J = 7.7 Hz) and 7.36 (*dd*, J = 1.5, 7.7 Hz). The chemical shifts and splitting patterns of these protons were similar to those of H-6, H-7 and H-8 of moscatin, respectively (Majumder and Sen, 1987). Two pairs of *ortho*-coupled protons appearing at δ7.15 (*d*, J = 9.1 Hz) and 6.95 (*d*, J = 9.1 Hz) and 7.39 (*d*, J = 9.1 Hz) and 7.34 (*d*, J = 9.1 Hz), were typical of H-9 /H-9′ and H-10/H-10′ of phenanthrenes. One pair of *meta*-coupled protons at δ7.01 (*d*, J = 2.5 Hz) and 7.02 (*d*, J = 2.5 Hz), one single aromatic proton at δ7.19 and another pair of *ortho*-coupled protons at δ7.26 (*d*, J = 7.7 Hz) and δ7.44 (*d*, J = 7.7 Hz) were also present. These observations, together with the previous data, indicated that **2** is, in fact, an asymmetric dimer of moscatin. Careful inspection of the NMR spectroscopic data indicated the possible linkage site should be at C-1 and C-1′, since the signals of H-1 and H-1′ (H-8 in moscatin) disappeared, changing C-1 and C-1′ from tertiary to quaternary carbons.

The linkage sites of the two monomers were further deduced by 2D NMR (HMBC) experiments. Cross-peaks were found between H-3 (δ7.19) and C-1 (δ119.1), H-10 (δ6.95) and C-1, H-2′ (δ7.44) and C-1, and between H-10′ (δ7.39) and C-1′ (δ121.3), establishing the 1,1′-coupling in **2**. On the basis of the above data, **2** was finally identified as 4,5′-dimethoxy-[1,1′]biphenanthrenyl-2,5,4′,7′-tetraol, named denthyrsinol, which is a new asymmetric dimeric phenanthrene derivative.

Compound **3** was obtained as a red amorphous powder, m.p. 348.2–349.1 °C. HREIMS of **3** exhibited a molecular ion peak at *m/z* 522.1287 $[M]^+$. The UV spectrum of **3** showed the absorption maxima at 213, 244, 262 and 318 nm, similar to other phenanthrene and phenanthraquinone derivatives (Majumder and Lahiri, 1990; Talapatra et al., 1982). The IR spectrum exhibited absorptions at 3401 (OH), 1650, 1642 (characteristic of chelated quinone) (Talapatra et al., 1982; Dominguez et al., 1976) and 1610, 1589, 828, 670 (aromatic rings). From the NMR (Table 3) and mass spectroscopic data, compound **3** is suggested to be a phenanthrene–phenanthraquinone derivative (Fan et al., 2001). The ^{1}H NMR spectrum exhibited signals for three pairs of *ortho*-coupled protons, with two pairs due to H-9/H-9′ and H-10/H-10′, one pair of *meta*-coupled protons, two single aromatic protons and three methoxyl groups. The *ortho*-coupled protons at δ9.45 (*dd*, J = 9.3, 0.6 Hz) and 7.26 (*d*, J = 9.3 Hz) were typical of H-5′ and H-6′ of a phenanthrene derivative (Majumder and Sen, 1987). The proton signal at δ7.76 (*dd*, J = 9.3, 0.6 Hz) was assigned to H-9′ due to the W-coupling with H-5′ (*dd*, J = 9.3, 0.6 Hz). HMBC cross-peaks (Fig. 2) were found between H-5′ (δ9.45) and C-7′ (δ147.1), and between H-9′ (δ7.76) and C-8′ (δ142.3), and between the methoxyl protons at δ3.93 and C-8′, indicating the presence of hydroxyl and methoxyl groups at C-7′ and C-8′, respectively. In addition, the methoxyl protons at δ3.91 showing a correlation with H-3′ but not with H-5′ in the NOESY spectrum (Fig. 2) implied a C-2′ substitution of a methoxyl. The remaining hydroxyl group can be assigned to C-4′. The absence of H-1′ and the correlations between H-10′ and C-1′, and between H-3′ and C-1′ indicated C-1′ to be the linkage site, indicating one part of the structure as 4′,7′-dihydroxy-2′,8′-dimethoxy-phenanthrenyl.

For the remaining part of the structure, IR and ^{13}C NMR spectra revealed two quinone carbonyls (δ184.0 and 188.4). The pair of *ortho*-coupled protons appearing at δ7.95 (*d*, J = 8.6 Hz) and 8.02 (*d*, J = 8.6 Hz) was typical of H-9 and H-10 of phenanthrene. In the HMBC

Table 3
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data of denthyrsinone (**3**) in acetone-d_6

Denthyrsinone (**3**)			
Carbon	^{1}H, δ (J, Hz)	^{13}C, δ	HMBC[a]
1		184.0	
2		160.2	
3	6.81 s	135.6	2, 4, 4a
4		188.4	
5		150.8	
6	6.80 (*d*, 2.1)	102.6	1′, 5, 7, 8
7		160.1	
8	6.95 (*d*, 2.1)	103.6	6, 7, 9, 4b, 8a
9	7.95 (*d*, 8.6)	131.7	8, 10, 4b, 8a, 10a
10	8.02 (*d*, 8.6)	122.4	1, 9, 4a, 8a, 10a
4a		130.9	
4b		117.1	
8a		137.1	
10a		140.5	
1′		113.0	
2′		156.2	
3′	7.11 *s*	99.9	1′, 2′, 4′, 4a′
4′		158.9	
5′	9.45 (*dd*, 9.3, 0.6)	125.7	7′, 4a′, 8a′
6′	7.26 (*d*, 9.3)	118.2	5′, 7′, 8′
7′		147.1	
8′		142.3	
9′	7.76 (*dd*, 9.3, 0.6)	125.4	8′, 10′, 4b′, 8a′, 10a′
10′	8.03 (*d*, 9.3)	122.8	1′, 9′, 4a′, 8a′, 10a′
4a′		115.5	
4b′		133.6	
8a′		127.4	
10a′		126.1	
OMe	3.93	61.6 (C-8′)	
	3.91	56.6 (C-2′)	
	3.77	56.2 (C-2)	

[a] Carbons that correlate with the proton resonance.

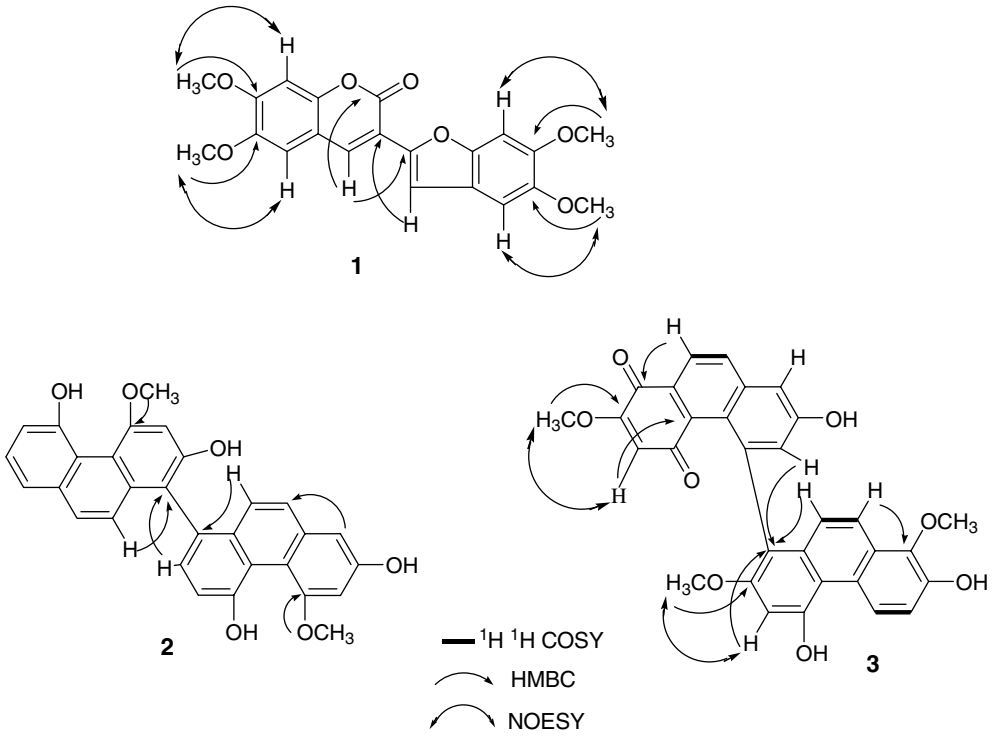

Fig. 2. Selected HMBC, COSY and NOESY correlations of denthyrsin (**1**), denthyrsinol (**2**) and denthyrsinone (**3**).

spectrum, cross peaks between H-10 and C-1 (δ184.0), H-3 (δ6.81) and C-4 (δ188.4), H-3 and C-4a (δ130.9), and H-3 and C-2 (δ160.2) indicated that C-1 and C-4 were carbonyls. Methoxyl signal at δ3.77 substituted on C-2 was also deduced from the HMBC cross peaks between methoxyl protons and C-2. In addition, correlations were found between H-9 and C-8 (δ103.6), H-8 and C-7 (δ160.1), and H-6 and C-7, indicating that the hydroxyl group is substituted on C-7. The absence of a downfield aromatic proton below δ8.0 attributed to H-5 implied C-5 should be the linkage site. Therefore, the segment was revealed as 7-hydroxy-2-methoxyphenanthrene-1,4-dione. In addition, a HMBC cross peak (Fig. 2) between H-6 and C-1′ further confirmed the linkage sites at C-5 and C-1′. Compound **3** was finally established as 7,4′,7′-trihydroxy-2,2′,8′-trimethoxy-[5,1′]biphenanthrenyl-1,4-dione, named denthyrsinone, a novel phenanthrene–phenanthraquinone class of compounds from natural resources.

1,5,7-Trimethoxyphenanthrene-2,6-diol (**4**, CAS Registry No. 118169-17-8) was previously reported by Majumder and Sen (1991) and improperly named as pendulin. In fact, pendulin has been given to compound 5-hydroxy-3,6,7-trimethoxyflavonol-4′-*O*-β-D-glucopyranoside (CAS Registry No. 14801-84-4) by Farkas et al. (1966). So, the common name of compound **4** should be revised and named as denthyrsinin in this paper.

The cytotoxicities of seven selected compounds from *D. thyrsiflorum* and erianin (positive control) against several tumor cell lines were determined by MTT assay. The results are shown in Table 4 as IC_{50} values.

Denthyrsin (**1**) is presumably derived from the biogenetically acceptable intermediate scoparone (**5**) which was also isolated from the same plant. Compound **1** showed more potential activities than **5** against Hela, K-562 and MCF-7 cell lines, indicating coumarins may be more active after coupling with benzofuran.

Denthyrsinol (**2**) is a novel asymmetric dimeric phenanthrene derivative and its co-occurrence with moscatin (**6**) and hircinol (**7**) in the same plant provides another strong circumstantial evidence for the role of oxidative coupling in the biogenesis of naturally occurring phenolic dimers from the corresponding monomeric phenols (Majumder and Banerjee, 1988). Denthyrsinol (**2**) has

Table 4
Cytotoxic activities of seven compounds *from D. thyrsiflorum*

Compound	IC_{50} (μM)		
	Hela	K-562	MCF-7
Denthyrsin (**1**)	13.5	0.45	18.1
Denthyrsinol (**2**)	9.3	1.6	–
Denthyrsinone (**3**)	9.9	6.0	3.5
Denthyrsinin (**4**)	2.7	2.3	4.8
Scoparone (**5**)	>100	>100	>100
Moscatin	>100	7.1	>100
Hircinol	>100	6.3	>100
Erianin	0.0071	0.0014	0.0017

potential activity while moscatin (**6**) and hircinol (**7**) were less effective in killing Hela and MCF-7 cells, which also indicated that the dimerization of phenanthrene plays an important role for the cytotoxicity against Hela and MCF-7 cell lines.

Denthyrsinin (**4**) and moscatin (**6**) are similar in structure, but denthyrsinin (**4**) showed higher cytotoxic activity against Hela and MCF-7 cells than moscatin (**6**) did. It suggests that the numbers and the substituted positions of methoxyl groups in phenanthrenes are important for the cytotoxic activity against Hela and MCF-7 cells.

A fairly large number of phenanthrenes and 9,10-dihydrophenanthrenes were reported from a series of orchids, e.g., *Dendrobium* (Lee et al., 1995), *Bulbophyllum* (Majumder et al., 1999), *Eria* (Bhandari et al., 1985), *Lusia* (Majumder and Lahiri, 1990), *Maxillaria* (Estrada et al., 1999) et al. Four symmetric dimeric phenanthrenes were reported early from Orchid *Cirrhopetalum maculosum* (Majumder et al., 1990), *Bulbophyllum reptans* (Majumder et al., 1999), *Agrostophyllum khasiyanum* and *Agrostophyllum callosum* (Majumder et al., 1998), and one asymmetric dimeric phenanthrene isolated from *B. reptans* (Majumder et al., 1999), as well as two dimeric 9,10-dihydrophenanthrenes from *Eria flava* (Majumder and Banerjee, 1988) and *Dendrobium plicatile* (Yamaki and Honda, 1996). Conclusively, numerous phenanthrenes and their dimeric derivatives have been found in higher plants, but the activities and the structure–function relationship of these type of compounds were rarely reported, and are worthy of future investigation. In addition, based on our preliminary biological assay, the dimers have stronger cytotoxicities than their monomers. Accordingly, more exhaustive studies should be conducted to disclose their biosynthetic pathways, and the key enzymes involved in dimerization of phenanthrenes, as well as to the discovery of other lead compounds as novel antitumor agents.

3. Experimental

3.1. General experimental procedures

UV spectra were measured on a Beckman DU-600 spectrophotometer. IR spectra were recorded on a Nicolet Magna 750 spectrophotometer with KBr discs. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a Bruker AMX-500 spectrometer in $CDCl_3$ (**1**) and acetone-d_6 (**2** and **3**). Chemical shifts are reported in ppm. ^{1}H NMR and ^{13}C NMR chemical shifts were referenced to solvent resonances at δ7.27 ($CDC1_3$) and δ2.05 (CD_3OCD_3) and δ77.2 ($CDCl_3$) and 206.5 (Acetone-d_6), respectively. EIMS and HREIMS were run on Shimamzu GCMS-QP5050A and Finigan MAT 95 spectrometers, respectively.

3.2. Plant material

D. thyrsiflorum Rchb.f was collected in Yunan Province, the People's Republic of China in October 2001, and authenticated by Professor Luo-Shan Xu. A voucher specimen of the plant (No. QH-0210) was deposited at the Department of Pharmacognosy, China Pharmaceutical University, Nanjing, the People's Republic of China.

3.3. Extraction and isolation

The air-dried stems of *D. thyrsiflorum* (14 kg) were broken into pieces and extracted with EtOH (3×50 L) at room temperature. Removal of EtOH under reduced pressure afforded a dark residue (700 g). The residue was suspended in water and partitioned with EtOAc and *n*-BuOH successively to give three fractions (EtOAc phase, 400 g, *n*-BuOH phase 90 g, and aqueous phase 150 g). An aliquot of the EtOAc-soluble fraction (300 g) was subjected to silica gel column chromatography, eluted with petroleum ether–EtOAc (100:1, 100:5, 100:10, 100:20, 100:40, 100:100) to yield six fractions (I–VI). Fractions I–V were further subjected to repeated column chromatography on silica gel eluted with petroleum ether–acetone (1:10–0:1) and on a Sephadex LH-20 column eluted with chloroform–methanol (1:1). Chrysophanol (58 mg), physicon (64 mg) and β-sitosterol (300 mg) were obtained from fraction II. Compound **1** (14 mg), **5** (188 mg), and emodin (69 mg) were obtained from fraction III. Compound **4** (18 mg) was obtained from fraction IV. Denthyrsinone **3** (16 mg) and daucosterol (260 mg) were obtained from fraction V.

The *n*-BuOH-soluble fraction was subjected to silica gel column chromatography, eluted with chloroform–methanol (100:1, 100:10, 100:20, 100:30, 100:50) to yield five fractions (I–V). An aliquot of Fraction III was passed through a Sephadex LH-20 column, eluted with methanol to remove pigments, and then further purified repeatedly by RP-18 column chromatography, eluted with MeOH–H_2O (7:3) to give **2** (12 mg).

3.3.1. 3-(5′,6′-Dimethoxy-benzofuran-2′-yl)- 6,7-dimethoxy-chromen-2-one (denthyrsin, *1*)

Yellow needles, m.p. 262.1–263.0 °C IR ν_{max} (KBr) 3086, 1717, 1618, 975, 618 cm^{-1}; UV λ_{max} (MeOH) (log ε) 263 (3.81), 286 (3.75), 318 (3.45), 337 (2.58) nm; HREIMS found *m/z* 382.1048 $[M]^+$, calculated for $C_{21}H_{18}O_7$ *m/z* 382.1053. For ^{1}H NMR and ^{13}C NMR spectroscopic data, see Table 1.

3.3.2. 4,5′-Dimethoxy-[1,1′]biphenanthrenyl-2,5,4′,7′-tetraol (denthyrsinol, *2*)

White amorphous powder, m.p. 361.3–362.9 °C; IR ν_{max} (KBr) 3401, 1589, 1533, 827, 659 cm^{-1}; UV λ_{max}

(MeOH) (log ε) 214 (4.52), 255 (4.36), 286 (4.19), 321 (3.85) nm; HREIMS found *m/z* 478.1424 $[M]^+$, calculated for $C_{30}H_{22}O_6$ *m/z* 478.1416. For ^1H NMR and ^{13}C NMR spectroscopic data, see Table 2.

3.3.3. 7,4′,7′-Trihydroxy-2,2′,8′-trimethoxy-[5,1′] biphenanthrenyl-1,4-dione (denthyrsinone, **3**)

Red amorphous powder, m.p. 348.2–349.1 °C; IR ν_{max} (KBr) 3401, 1610, 1589, 828, 670 cm^{-1}; UV λ_{max} (MeOH) (log ε) 213 (4.55), 244 (4.36), 262 (4.31), 318 (3.98) nm; HREIMS found *m/z* 522.1287 $[M]^+$, calculated for $C_{31}H_{22}O_8$ *m/z* 522.1325. For ^1H NMR and ^{13}C NMR spectroscopic data, Table 2.

3.4. Cytotoxic studies

The tumor cells were maintained in RPMI-1640 (Hela) and RPMI-DMEM (K-562, MCF-7) supplemented with 10% fetal bovine serum. The cell cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2. The cell growth was evaluated by MTT assay as previously reported (Mosmann, 1983). The tumor cells were treated with seven selected compounds from *D. thyrsiflorum* and erianin (positive control) for 72 h. The results are expressed as IC_{50} in μM, i.e., concentration of test samples to give 50% inhibition of the growth of tumor cells.

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