A New Anthraquinone and Two New Tetrahydroanthraquinones from the Roots of *Prismatomeris connata*

by **Shi-Xiu Feng**[a])[b]), **Jing Hao**[a]), **Tao Chen***[b]), and **Samuel X. (Shengxiang) Qiu***[a])

[a]) Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, 723 Xingke Road, Tianhe District, Guangzhou, 510650, P. R. China

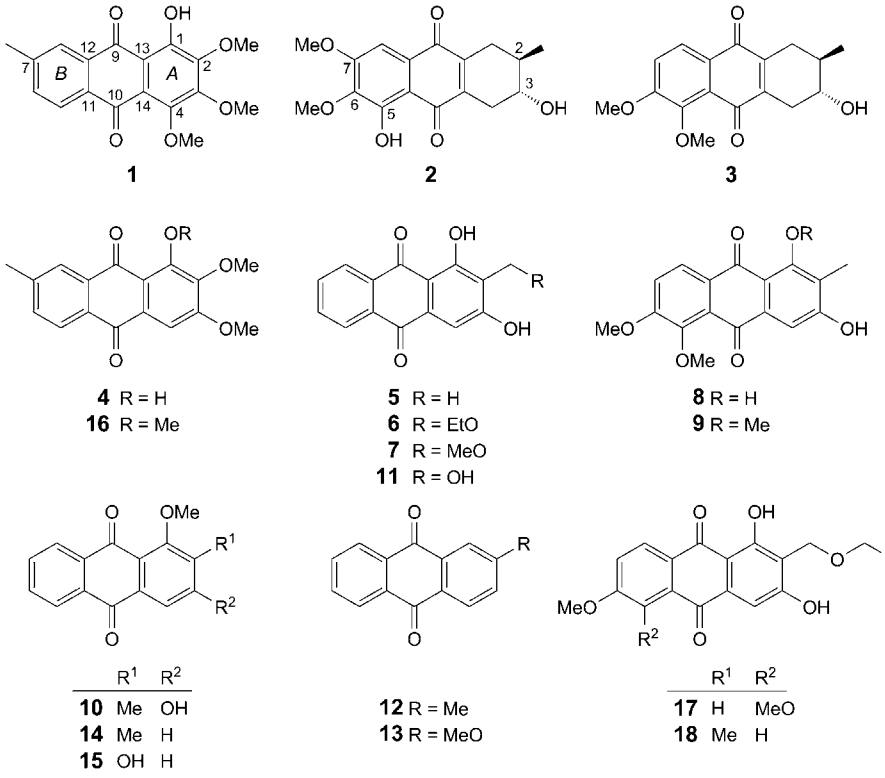
[b]) Laboratory of Southern Subtropical Plant Diversity, Shenzhen Fairy Lake Botanical Garden, Chinese Academy of Sciences, 160 Xianhu Road, Liantang, Luohu District, Shenzhen, 518004, P. R. China (phone/fax: +86-20-37081190; e-mail: taochen.mobg@gmail.com; sxqiu@scbg.ac.cn)

A new anthraquinone, 4-hydroxy-1,2,3-trimethoxy-6-methylanthracene-9,10-dione (**1**), and two novel tetrahydroanthraquinones, prisconnatanones A and B (**2** and **3**, resp.), together with 15 known anthraquinones, **4**–**18**, were isolated from the roots of *Prismatomeris connata*. Their structures were elucidated on the basis of spectroscopic data. Compounds **1**–**18** were evaluated for their cytotoxicity against A549 and LAC human cancer cell lines. Compound **2** and the anthraquinones with free OH groups exhibited activity against the test cell lines.

Introduction. – *Prismatomeris* (Rubiaceae), a genus comprising *ca.* 16 species, is distributed in the tropical and subtropical areas of the world. Of this genus, only one species and two subspecies are native to China [1]. *P. tetrandra* ssp. *multiflora* is distributed in Yunnan of China, whereas *P. connata* and *P. connata* ssp. *hainanensis* grow in Fujian, Guangdong, Guangxi, and Hainan. The roots of these species, all called '*Huanggen*', are used as a Chinese folk medicine for the treatment of hepatitis, anaemia, leucocythemia, and pneumoconiosis [2–4]. Previous phytochemical investigation of this genus led to the isolation of cytotoxic anthraquinones and iridoids [5–7]. In continuation of our phytochemical studies on bioactive anthraquinones in the family of Rubiaceae, the roots of *P. connata* were investigated, leading to the isolation and structure elucidation of a new anthraquinone, 4-hydroxy-1,2,3-trimethoxy-6-methylanthracene-9,10-dione (**1**), and two new rare tetrahydroanthraquinones, trivially named prisconnatanones A and B (**2** and **3**, resp.), together with 15 known anthraquinones, **4**–**18**. In addition, the cytotoxicities of the isolated compounds, **1**–**18**, were evaluated against A549 and LAC human cell lines. Here, we report the isolation, structure elucidation, and cytotoxicity evaluation of these compounds. All compounds, **1**–**18**, were reported for the first time from this species.

Results and Discussion. – The EtOH extract of the roots of *P. connata* was fractionated with petroleum ether (PE), AcOEt, and BuOH. The PE-soluble fraction was subjected to repeated column chromatography on silica gel, *Sephadex LH-20*, and *ODS* to afford three new compounds, **1**–**3**, along with the known anthraquinones, 1-hydroxy-2,3-dimethoxy-7-methyl-9,10-anthraquinone (**4**) [7], 1,3-dihydroxy-2-methyl-

© 2011 Verlag Helvetica Chimica Acta AG, Zürich



anthraquinone (**5**) [6], ibericin (**6**), lucidin ω-methyl ether (**7**) [8], 1,3-dihydroxy-5,6-dimethoxy-2-methyl-9,10-anthraquinone (**8**), 3-hydroxy-1,5,6-trimethoxy-2-methyl-9,10-anthraquinone (**9**) [7], 3-hydroxy-1-methoxy-2-methyl-9,10-anthraquinone (**10**), 2-methylanthraquinone (**12**) [9], 1,3-dihydroxy-2-(hydroxymethyl)-9,10-anthraquinone (**11**) [8], 2-methoxyanthraquinone (**13**), 1-methoxy-2-methylanthraquinone (**14**) [10], 2-hydroxy-1-methoxyanthraquinone (**15**) [11], 1,2,3-trimethoxy-7-methylanthraquinone (**16**) [12], 1,3-dihydroxy-5,6-dimethoxy-2-(methoxymethyl)-9,10-anthraquinone (**17**) [13], and 6-methoxyibericin (**18**) [8]. The structures of the known compounds were determined by interpretation of their spectroscopic data as well as by comparison with reported data.

Compound **1** was obtained as a yellow power. Its molecular formula was determined as $C_{18}H_{16}O_6$ from a *quasi*-molecular-ion peak at *m/z* 329.1020 ($[M+H]^+$, $C_{18}H_{17}O_6^+$; Δ +0.138 ppm) in the HR-ESI-MS. The IR spectrum of **1** showed the presence of OH (3444 cm^{-1}) and conjugated CO groups (1671 cm^{-1}), and aromatic rings (1575, 1461 cm^{-1}). The UV spectrum exhibited absorption maxima at 208, 267, and 416 nm, attributable to an anthraquinone chromophore [7]. The ^{1}H-NMR spectrum displayed a *singlet* at δ(H) 13.69 (*s*, 1 H) for a chelated phenolic OH group and an *ABX* system at δ(H) 8.13 (*d*, $J=8.0$, 1 H), 7.59 (*dd*, $J=8.0$, 2.0, 1 H), and 8.00 (*d*, $J=2.0$, 1 H) for a 1,3,4-trisubstituted benzene ring. The spectrum also exhibited

signals for a benzylic Me group (δ(H) 2.50 (*s*, Me–C(7))), three aromatic MeO groups (δ(H) 4.06 (*s*, MeO–C(3) and MeO–C(4)), 3.93 (*s*, MeO–C(2))). In the ^{13}C-NMR spectrum, besides 14 C-atom signals assignable to the anthraquinone nucleus, resonances for one benzylic Me and three MeO groups were observed. Thus, an anthraquinone with five substituents was deduced, in which one of the rings was mono-substituted and the other was tetra-substituted. In the HMBC spectrum (*Fig. 1*), long-range correlations of the aromatic H–C(8) (δ(H) 8.00) with both the C(9)=O and the benzylic Me–C(7) were observed, indicating of the location of the benzylic Me group at C(7). The presence of HMBCs from the chelated phenolic HO–C(1) (δ(H) 13.69) to the C-atoms at C(13) and C(2) indicated location of this phenolic OH group at C(1). The three MeO groups were at C(2), C(3), and C(4). Therefore, the structure of **1** was elucidated as 4-hydroxy-1,2,3-trimethoxy-6-methylanthracene-9,10-dione.

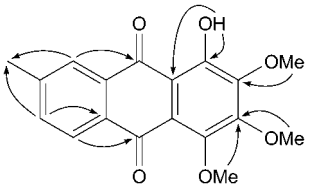


Fig. 1. *Key HMBCs of* **1**

Compound **2** was obtained as a yellow power. The molecular formula was determined as $C_{17}H_{18}O_6$ from the HR-ESI-MS, which gave a *pseudo*-molecular-ion peak at *m/z* 341.0995 ($[M+Na]^+$, $C_{17}H_{18}NaO_6^+$; Δ −0.116 ppm). The IR spectrum showed the presence of OH (3488 cm^{-1}) and conjugated CO groups (1637 cm^{-1}), and aromatic rings (1610, 1589, and 1498 cm^{-1}). The UV spectrum exhibited absorptions at 218, 265, 290, and 414 nm, in accordance with a naphthoquinone chromophore [14]. The ^{1}H- and ^{13}C-NMR spectra (*Table 1*) revealed the presence of a phenolic OH group (δ(H) 12.26 (*s*, HO–C(5))), two aromatic MeO groups (δ(H) 3.99 (*s*, MeO–C(6)) and 4.00 (*s*, MeO–C(7))), two conjugated ketone CO groups (δ(C) 178.5 (C(9)) and 184.1 (C(10))), an aromatic CH group (δ(H) 7.25 (*s*, H–C(8)), δ(C) 99.0 (C(8))), and seven aromatic quaternary C-atoms. These structural features were in accordance with a pentasubstituted nephthoquinone. Furthermore, the spectra exhibited signals indicating the presence of two benzylic CH_2 groups (δ(H) 2.94 (*dd*, J = 19.0, 5.6, H_{eq}–C(1)), 2.30 (*dd*, J = 19.0, 7.9, H_{ax}–C(1)), 2.53 (*dd*, J = 19.0, 8.1, H_{ax}–C(4)), and 3.03 (*dd*, J = 19.0, 4.8, H_{eq}–C(4)); δ(C) 24.1 (C(1)), 26.2 (C(4))), an O-bearing CH group (δ(H) 3.74 (*td*, J = 8.1, 4.8, H–C(3)); δ(C) 65.8 (C(3))), a secondary Me group (δ(H) 1.12 (*d*, J = 6.8); δ(C) 12.6 (Me–C(2))), and a CH group (δ(H) 1.92–1.96 (*m*, H–C(2)); δ(C) 28.9 (C(2))). The COSY spectrum together with HSQC spectrum revealed the connectivity from C(1) to C(4) with the secondary Me group attached to C(2) to form a 3-hydroxy-2-methylbutane moiety (*Fig. 2*). In the HMBC spectrum (*Fig. 2*), correlations from CH_2(1) to C(13), C(9), and C(14), and from CH_2(4) to C(13) and C(10) were observed, indicating that the 3-hydroxy-2-methylbutane moiety was connected to the naphthoquinone ring by the linkages of C(1) with C(13), and of C(4) with C(14) to form a 1,2,3,4-tetrahydroanthraquinone skeleton. HMBCs were also observed from H–C(8) to C(9), C(11), and C(6), and from HO–C(5) to C(5), C(6), and C(11),

Table 1. *^{1}H- and ^{13}C-NMR Data of Compounds* **2** *and* **3** *in $CDCl_3$* (J in Hz)

Position	**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)
1_{eq}	2.94 (*dd*, J = 19.0, 5.6)	24.1	2.89 (*dd*, J = 19.4, 5.4)	29.2
1_{ax}	2.30 (*dd*, J = 19.0, 7.9)		2.29 (*dd*, J = 19.4, 8.0)	
2	1.92 – 1.96 (*m*)	28.9	1.87 – 1.90 (*m*)	33.6
3	3.74 (*td*, J = 8.1, 4.8)	65.8	3.72 (*td*, J = 8.0, 4.7)	70.9
4_{eq}	3.03 (*dd*, J = 19.0, 4.8)	26.2	3.01 (*dd*, J = 19.2, 4.7)	31.0
4_{ax}	2.53 (*dd*, J = 19.0, 8.1)		2.52 (*dd*, J = 19.2, 8.0)	
5		150.8		149.0
6		136.5		158.6
7		152.9	7.15 (*d*, J = 8.0)	115.3
8	7.25 (*s*)	99.0	7.90 (*d*, J = 8.0)	124.2
9		178.5		183.9
10		184.1		183.6
11		106.1		125.5
12		123.0		126.0
13		138.7		143.2
14		138.5		141.9
2-Me	1.12 (*d*, J = 6.8)	12.6	1.10 (*d*, J = 6.8)	17.2
5-MeO			3.96 (*s*)	61.1
6-MeO	3.99 (*s*)	56.3	3.92 (*s*)	56.2
7-MeO	4.00 (*s*)	51.7		
5-OH	12.26 (*s*)			

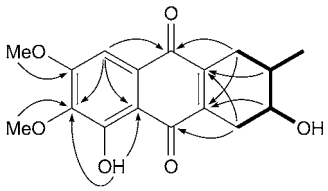


Fig. 2. *^{1}H,^{1}H-COSY* (bold lines) *and key HMBC* (arrows) *correlations of* **2**

indicating that C(8) was unsubstituted, and the OH group was attached to C(5). Further, the two MeO groups were readily located at C(6) and C(7). Based on these evidences, the planar structure of **2** was deduced as 1,2,3,4-tetrahydro-3,5-dihydroxy-6,7-dimethoxy-2-methylanthracene-9,10-dione.

The relative configuration of **2** was determined by the NOESY experiment and the H-atom coupling constants. In the NOESY spectrum, NOE correlations between H–C(2) and H_{ax}–C(4), and between H–C(3) and H_{ax}–C(1) were observed (*Fig. 3*). This, in combination with the axial-axial coupling constant values (J = 7.9 or 8.1) observed between H_{ax}–C(1) and H–C(2), H–C(2) and H–C(3), and H–C(3) and H_{ax}–C(4) indicated a half-chair form of the tetrahydrobenzene ring with both Me–C(2) and HO–C(3) in equatorial positions. Therefore, the structure of **2**, trivially named prisconnatanone A, was established as 1,2,3,4-tetrahydro-3α,5-dihydroxy-6,7-dimethoxy-2β-methylanthracene-9,10-dione.

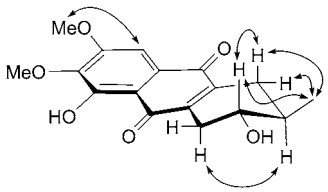


Fig. 3. *Key NOESY correlations of* **2**

Compound **3** was also obtained as a yellow power. Its molecular formula was determined as $C_{17}H_{18}O_5$ by combined analysis of the ESI-MS and HR-ESI-MS data (325.1046 ($[M+Na]^+$,$C_{17}H_{18}NaO_5^+$; Δ −0.076 ppm)), indicating that **3** had one O-atom less than **2**. The ^{1}H- and ^{13}C-NMR spectra were similar to those of **2** except the absence of the H-atom signal for HO–C(5). Instead, the spectra exhibited signals (δ(H) 7.15 (*d*, *J* = 8.0, 1 H), 7.90 (*d*, *J* = 8.0, 1 H); δ(C) 115.3 (C(7)), 124.2 (C(8))), suggesting that C(7) in **3** was unsubstituted, and HO–C(5) in **2** was methylated in **3**. By analysis of 2D-NMR spectra (^{1}H,^{1}H-COSY, HSQC, HMBC, and NOESY), the ^{1}H- and ^{13}C-NMR spectroscopic data were assigned as compiled in *Table 1*, and fully supported the structure. Thus, the structure of **3**, named prisconnatanone B, was established as 1,2,3,4-tetrahydro-3α-hydroxy-5,6-dimethoxy-2β-methylanthracene-9,10-dione.

The cytotoxicity of the three new compounds, **1**–**3**, as well as 15 known anthraquinones, **4**–**18**, were all evaluated against human lung cancer (A549) and human pulmonary carcinoma (LAC) cell lines using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) colorimetric assay. As can been seen in *Table 2*, the tetrahydroanthraquinone **2** exhibited potent cytotoxicity against the tested cell lines with IC_{50} values of 4.5 and 7.8 μM, respectively, while compound **3** was almost inactive against both cell lines. Among the anthraquinones, compounds **1**,

Table 2. *Cytotoxicity of Compounds* **1**–**18**[a]) *by the MTT Assay* (IC_{50} [μM])

	Cell lines	
	A549	LAC
2	4.5 ± 0.1	7.8 ± 0.1
3	> 100	98.7 ± 0.5
4	> 100	67.3 ± 0.2
5	> 100	17.0 ± 0.1
6	23.8 ± 0.5	60.3 ± 0.6
7	44.6 ± 0.3	34.9 ± 0.3
8	93.6 ± 1.1	36.8 ± 0.2
9	28.9 ± 0.6	9.6 ± 0.2
10	> 100	38.5 ± 0.5
12	16.1 ± 0.4	22.5 ± 0.3
17	65.2 ± 1.4	99.1 ± 1.0
18	55.5 ± 0.6	25.3 ± 0.2
Doxorubicin[b])	14.2 ± 0.1	10.5 ± 0.1

[a]) Compounds **1**, **11**, and **13**–**16** were inactive (IC_{50} > 100 μM) against the tested cell lines. [b]) Positive control.

11, **13**–**16** exhibited no significant cytotoxicity ($IC_{50} > 100$ μM) against the two tumor cell lines. Compounds **6**–**9**, **12**, **17**, and **18** showed inhibitory effects against A549 cell line with IC_{50} values ranging from 16.1 to 93.6 μM, and compounds **4**–**10**, **12**, **17**, and **18** exhibited activities against LAC cell line with IC_{50} values ranging from 9.6 to 99.1 μM. The activity profiles suggest that the phenolic OH group might be necessary for the antitumor potency of tetrahydroanthraquinones and anthraquinones.

This work was supported by the *Natural Science Foundation of China* (No. 30973635) and the *Shenzhen Urban Management Bureau Grant* (Chen Tao 2006).

Experimental Part

General. Column chromatography (CC): silica gel *60* (SiO_2, 100–200 and 200–300 mesh, *Qingdao Marine Chemical Ltd.*, Qingdao, P. R. China), *C-18* reversed-phase (RP) silica gel (40 and 70 μm, *J. T. Baker*, USA), and *Sephadex LH-20*. M.p.: *Yanagimoto Seisakusho MD-S2*; uncorrected. Optical rotations: *Perkin-Elmer 341* polarimeter with MeOH as solvent. UV Spectra: in MeOH on a *Perkin-Elmer Lambda 25* UV/VIS spectrophotometer. IR Spectra: in KBr on a *WQF-410 FT-IR* spectrophotometer. ^{1}H- (400 MHz), ^{13}C- (100 MHz), and 2D-NMR spectra: *Bruker DRX-400* instrument; TMS as an internal standard, in $CDCl_3$. ESI-MS: *MDS SCIEX API 2000 LC/GC/MS* instrument. HR-ESI-MS: *Bruker Bio-TOF-IIIQ* mass spectrometer.

Plant Material. The roots of *P. connata* (50 kg) were collected from Nanning, Guangxi Province, P. R. China, in July 2008 and identified by *T. C.* (Shenzhen Fairy Lake Botanical Garden). An authenticated voucher specimen (No. 320959) was deposited with the Herbarium of South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, P. R. China.

Extraction and Isolation. The air-dried roots of *P. connata* (50 kg) were powdered and extracted with 95% EtOH (3 × 80 l) at r.t. The extract (910 g) was suspended in H_2O (1200 ml) and partitioned with petroleum ether (PE; 60–90°; 4000 ml), AcOEt (4000 ml), and BuOH (3000 ml), successively. The PE-soluble extract (80 g) was subjected to CC (SiO_2; PE/acetone 10:1 to 3:1) to yield eight fractions, *Frs. 1–8*. *Fr. 2* (3.0 g) was further separated by CC (*ODS*; 75% MeOH) to obtain six subfractions, *Frs. 2a–2f*. *Fr. 2c* (250 mg) was purified by CC (*Sephadex LH-20*; MeOH) to give compounds **4** (23 mg) and **16** (48 mg). *Fr. 2d* (150 mg) was subjected to recrystallization from PE/acetone 3:1 to afford compound **6** (56 mg). *Fr. 2e* (300 mg) was separated by CC (*ODS*; 75% MeOH) to obtain compounds **1** (15 mg) and **14** (55 mg). *Fr. 2f* (120 mg) was purified by CC (*Sephadex LH-20*; MeOH) to yield compound **17** (25 mg). *Fr. 3* (2.8 g) was submitted further to CC (*ODS*; 70% MeOH) to afford four subfractions, *Frs. 3a–3d*. *Frs. 3c* (650 mg) and *3d* (550 mg) were separated by CC (*Sephadex LH-20*; MeOH), followed by recrystallization from PE/acetone 3:1, to yield compounds **5** (93 mg) and **7** (110 mg), resp. *Fr. 5* (920 mg) was subjected to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:3) to obtain five subfractions, *Frs. 5a–5e*. *Fr. 5c* (420 mg) was further purified by CC (SiO_2; PE/acetone 3:1) to afford compounds **8** (90 mg) and **10** (120 mg). *Fr. 6* (3.6 g) was further subjected to CC (*ODS*; aq. MeOH of decreasing polarities (from 60 to 80%)) to give six subfractions, *Frs. 6a–6f*. *Fr. 6b* (150 mg) was purified by CC (*Sephadex LH-20*; MeOH) to afford compound **11** (18 mg). *Fr. 6c* (110 mg) was further subjected to CC (*ODS*; aq. MeOH) (from 70 to 80%) to provide compound **12** (15 mg). *Fr. 6d* (80 mg) was separated by CC (*ODS*; 70% MeOH) to obtain compound **13** (13 mg). *Fr. 6e* (100 mg) was further separated by CC (*ODS*; 65% MeOH) to yield compounds **18** (11 mg) and **15** (8 mg). *Fr. 7* (2.1 g) was separated by CC (*ODS*; 70% MeOH) to afford five subfractions, *Frs. 7a–7e*. *Fr. 7b* (80 mg) was purified by CC (*Sephadex LH-20*; MeOH) to yield compound **9** (18 mg). *Fr. 7c* (150 mg) was purified by the same method to yield compounds **3** (13 mg) and **2** (20 mg).

4-Hydroxy-1,2,3-trimethoxy-6-methylanthracene-9,10-dione (**1**). Yellow power. M.p. 165–167°. UV (MeOH): 207.9 (4.30), 267.0 (4.40), 416 (3.95). IR (KBr): 3444, 2979, 1671, 1575, 1461, 1284, 1249, 1159, 927. ^{1}H-NMR: 13.69 (*s*, HO–C(1)); 8.13 (*d*, $J = 8.0$, H–C(5)); 8.00 (*d*, $J = 2.0$, H–C(8)); 7.59 (*dd*, $J = 8.0$, 2.0, H–C(6)); 4.06 (*s*, MeO–C(3), MeO–C(4)); 3.93 (*s*, MeO–C(2)); 2.50 (*s*, Me–C(7)). ^{13}C-NMR: 188.0

(C(9)); 181.0 (C(10)); 155.5 (C(4)); 155.4 (C(2)); 149.1 (C(3)); 146.3 (C(1)); 146.0 (C(7)); 135.8 (C(6)); 134.8 (C(12)); 132.6 (C(11)); 127.5 (C(5)); 126.4 (C(8)); 120.4 (C(14)); 111.8 (C(13)); 61.4, 61.5 (*Me*O–C(2), *Me*O–C(3), *Me*O–C(4)); 21.5 (*Me*–C(7)). ESI-MS: 679.0 ($[2M+Na]^+$), 327.0 ($[M-H]^-$). HR-ESI-MS: 329.1020 ($[M+H]^+$, $C_{18}H_{17}O_6^+$; calc. 329.1025).

Prisconnatanone A (*=1,2,3,4-Tetrahydro-3α,5-dihydroxy-6,7-dimethoxy-2β-methylanthracene-9,10-dione*; **2**). Yellow power. M.p. 265–267°. $[\alpha]_D^{20} = -92$ ($c = 0.6$, MeOH). UV (MeOH): 217.5 (4.39), 265.0 (4.24), 290.9 (3.96), 413.9 (3.84). IR (KBr): 3488, 2975, 1637, 1610, 1589, 1498, 1457, 1421, 1319, 1286, 1141, 1062. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 341 ($[M+Na]^+$), 659 ($[2M+Na]^+$), 317 ($[M-H]^-$), 352.3 ($[M+Cl]^-$). HR-ESI-MS: 341.0995 ($[M+Na]^+$, $C_{17}H_{18}NaO_6^+$, calc. 341.1001).

Prisconnatanone B (*=1,2,3,4-Tetrahydro-3α-hydroxy-5,6-dimethoxy-2β-methylanthracene-9,10-dione*; **3**). Yellow power. M.p. 226–228°. $[\alpha]_D^{20} = -120$ ($c = 1.0$, MeOH). UV (MeOH): 212.5 (4.05), 263.6 (3.01), 386.3 (3.39). IR (KBr): 3513, 2927, 1654, 1569, 1334, 1267, 1068. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 303 ($[M+H]^+$), 325 ($[M+Na]^+$), 341 ($[M+K]^+$), 605 ($[2M+H]^+$), 627 ($[2M+Na]^+$), 301 ($[M-H]^-$), 639.4 ($[2M+Cl]^-$). HR-ESI-MS: 325.1046 ($[M+Na]^+$, $C_{17}H_{18}NaO_5^+$; calc. 325.1052).

Cytotoxicity Assay. Cytotoxicity of compounds **1**–**18** was determined by MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) method using human lung cancer (A549) and human pulmonary carcinoma (LAC) cell lines [15]. Human cancer cells were plated at 1×10^4 cell/well in 96-well microtiter plates and incubated for 24 h at 37° and 5% CO_2. Then, the cells were treated in triplicate with or without various concentrations of test samples. After 3 d of incubation at 37° and 5% CO_2, MTT reagent (5 mg/ml) was added to each well for 4 h. The resulting crystals were dissolved in DMSO (150 µl) and shaken for another 15 min. The absorbance was then determined by a (*TECAN Gennios*) at a wavelength of 570 nm. The inhibition percentages were calculated from reduction of absorbance in the control which was treated with 1% DMSO alone. Control wells received only the media without the test samples. The anticancer drug doxorubicin was used as positive control. The assay was performed in triplicate, and the data were presented as mean ± S.D.

REFERENCES

[1] J. T. Johansson, *Opera Bot.* **1987**, *94*, 5.
[2] Y.-Z. Ruan, *J. Syst. Evol.* **1988**, *26*, 443.
[3] 'Compendium of Chinese Traditional Herbal Drugs', People's Health Press, Beijing, 1992, Vol. 2, p. 772.
[4] C. Y. Zhou, J. G. Deng, *J. Guangxi Tradit. Chin. Med. Univ.* **2006**, *9*, 90.
[5] K. Krohn, D. Gehle, S. K. Dey, N. Nahar, M. Mosihuzzaman, N. Sultana, M. H. Sohrab, P. J. Stephens, J.-J. Pan, F. Sasse, *J. Nat. Prod.* **2007**, *70*, 1339.
[6] J. S. Jiang, Z. M. Feng, P. C. Zhang, *China J. Chin. Mater. Med.* **2005**, *30*, 1751.
[7] Z.-M. Feng, J.-S. Jiang, Y.-H. Wang, P.-C. Zhang, *Chem. Pharm. Bull.* **2005**, *53*, 1330.
[8] A. A. El-Gamal, K. Tankeya, H. Itokawa, A. F. Halim, M. M. Amer, A. S. Hassan-Elrady, S. A. Awad, *Phytochemistry* **1995**, *40*, 245.
[9] D. J. Tu, Z. H. Pang, N. J. Bi, *Acta Pharm. Sin.* **1981**, *16*, 631.
[10] A. R. Burnett, R. H. Thomson, *Phytochemistry* **1968**, *7*, 1421.
[11] V. V. S. Murti, T. R. Seshadri, S. Sivakumaran, *Phytochemistry* **1972**, *11*, 1524.
[12] D. A. Benson, I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, B. A. Rapp, D. L. Wheeler, *Nucleic Acids Res.* **2000**, *28*, 15.
[13] P. Tuntiwachwuttikul, Y. Butsuri, P. Sukkoet, U. Prawat, W. C. Taylor, *Nat. Prod. Res.* **2008**, *22*, 962.
[14] R. Suemitsu, Y. Yamada, T. Sano, K. Yamashita, *Agric. Biol. Chem.* **1984**, *48*, 2383.
[15] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55.

Received March 11, 2011