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Five new benzylphenanthrenes from *Cremastra appendiculata*

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ABSTRACT

Five new benzylphenanthrenes, cremaphenanthrenes L–P (**1**–**5**) were isolated from the EtOAc-soluble extract of the tubers of *Cremastra appendiculata*. Their structures were elucidated based on the spectroscopic data and chemical methods. Compounds **1**–**5** were tested in vitro for their cytotoxic effects against colon (HCT-116), cervix (Hela), and breast (MCF-7 and MDA-MB-231) cancer cell lines. Compound **1** showed moderate cytotoxic activities against HCT-116, MCF-7, and MDA-MB-231 cancer cell lines with IC_{50} values ranging from 15.84 to 24.18 μM and weak cytotoxicity to Hela cell line with IC_{50} value of 68.81 μM.

1. Introduction

The tuber of *Cremastra appendiculata* (D. Don) Makino (Orchidaceae), Shancigu in Chinese, is a famous traditional Chinese medicine with a long history for treating cancers [1]. Previous phytochemical investigations suggested that phenanthrenes were the main constituents in this plant, which have been reported to possess cytotoxic activities in vitro [2–5]. As a continuing program to search for cytotoxic phenanthrenes from *C. appendiculata*, UV-directed fractionation was carried out to isolate phenanthrenes from the EtOAc-soluble extract of this plant, which led to the isolation of five new benzylphenanthrenes, namely cremaphenanthrenes L–P (**1**–**5**) (Fig. 1). In this paper, we report the isolation and structural elucidation as well as the cytotoxic activities of five new benzylphenanthrenes.

2. Experimental

2.1. General experimental procedures

UV spectra were run on a Shimadzu UV-2450 spectrometer. IR spectra were recorded on a Nicolet NEXUS-470 FTIR spectrometer. Optical rotations were measured on a Rudolph AUTOPOL IV polarimeter. HR-ESI-MS was determined by a Bruker APEX IVFT-MS (7.0 T) and a Waters Xevo G2 Q-TOF/YCA mass spectrometers. NMR spectra were recorded on a Varian Inova-500 and a Bruker Avance-600 FT NMR spectrometers. Semi-preparative HPLC was run on a Dionex Ultimate 3000 instrument equipped with 170U UV Detector and an ODS column (Thermo Scientific BDS-C18 column, 250 mm × 10 mm, 5 μm). GC analyses were performed on a VARIAN CP-3800 instrument (DB-5, 0.25 mm × 30 m). Column chromatography (CC) was performed using silica gel (200–300 mesh, Qingdao Marine Chemistry Ltd., China), Sephadex LH-20 (Amersham Biosciences, Sweden) and ODS C_{18} (40–63 μm, Merck, Germany). TLC was carried out on glass precoated silica gel (GF_{254}) plates. Spots were visualized under UV light and detected by spraying with 10% H_2SO_4 in EtOH followed by heating.

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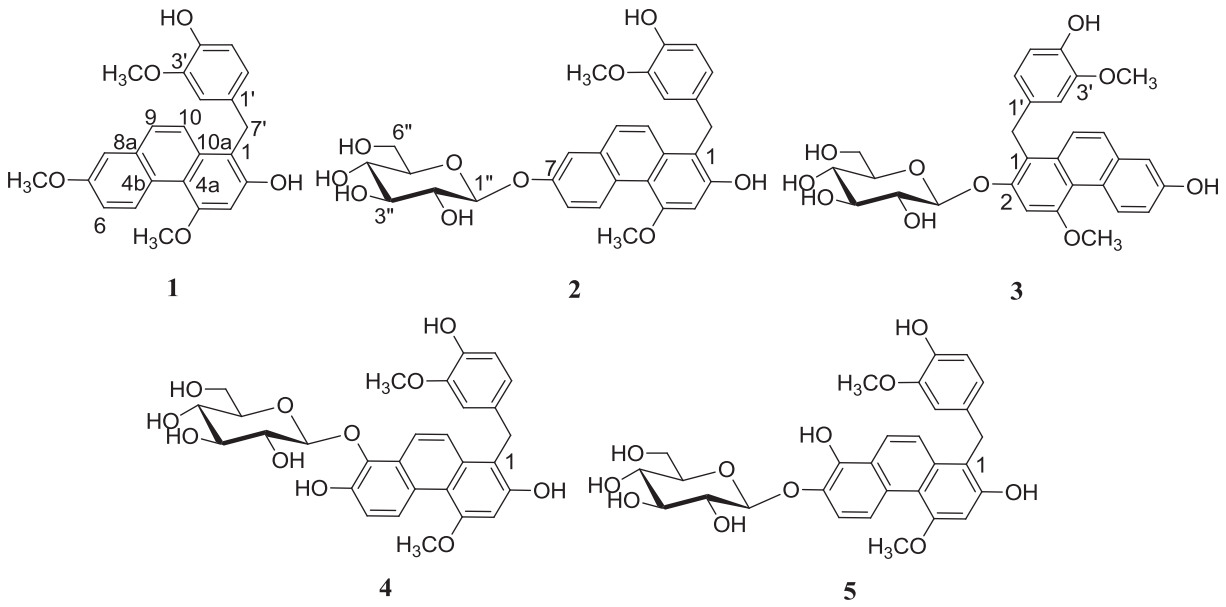

Fig. 1. Chemical structures of compounds **1**–**5**.

2.2. Plant material

The tubers of *C. appendiculata* were collected in Yunnan Province in June 2011. The plant materials were identified by one of the authors (Prof. P.F. Tu), and a voucher specimen (No. DJL20110628) was deposited at the Herbarium of Peking University Modern Research Center for Traditional Chinese Medicine.

2.3. Extraction and isolation

The powdered, dry tubers of *C. appendiculata* (30 kg) were extracted with 95% EtOH (100 L) three times. After filtration and evaporation, a dark brown residue was obtained. The residue was suspended in distilled water and partitioned successively with petroleum ether (PE, 3×10 L), EtOAc (3×10 L), and *n*-BuOH (3×15 L). The EtOAc fraction was separated by a silica gel CC (200–300 mesh) eluting with a gradient of $CHCl_3$:MeOH (50:1–0:100, v/v) to give 9 fractions (Fr. A–I) based on TLC analysis. Fr. A (21.6 g) was chromatographed on a silica gel column using a PE:acetone (5:2–0:100) gradient to get 13 fractions (Fr. A1–A13). Fr. A9 (1.8 g) was subjected to CC on a Sephadex LH-20 eluting with $CHCl_3$:MeOH (2:1) to yield 10 fractions (A9-1 to A9-10). Fr. A9-9 (58.5 mg) was purified by semi-preparative HPLC, washing with MeOH:H_2O (7:3, 2.0 mL/min) as mobile phase to yield compound **1** (3.5 mg). Fr. G (5.2 g) was applied to a RP-C18 CC eluting with a MeOH–H_2O (1:1–100:0) gradient to afford 10 fractions (Fr. G1–G10). Fr. G5 (1.34 g) was further purified on a Sephadex LH-20 using MeOH to give 11 fractions (Fr. G5-1 to G5-11). Fr. G5-8 (75.0 mg) was purified by semi-preparative HPLC, using CH_3CN–H_2O (1:4, 2.0 mL/min) as mobile phase, to yield compound **2** (5.4 mg) and **3** (8.8 mg). Fr. G5-9 (35.3 mg) and Fr. G5-11 (24.1 mg) was respectively purified by semi-preparative HPLC, both eluting with CH_3CN–H_2O (6:19, 2.0 mL/min) as mobile phase, to obtain compound **4** (2.3 mg) and compound **5** (2.0 mg), respectively.

2.3.1. Cremaphenanthrene L (*1*)

Brown amorphous powder; IR (KBr) ν_{max} (cm^{-1}) 3356, 2934, 1611, 1514, 1454, 1368, 1277, and 1230; UV λ_{max} (MeOH) 203 (0.75) and 264 (0.68) nm; negative ion HR-ESI-MS *m/z* 389.1398 $[M-H]^-$ (calcd for $C_{24}H_{21}O_5$, 389.1395); ^1H NMR and ^{13}C NMR data: Tables 1 and 2.

2.3.2. Cremaphenanthrene M (*2*)

Brown amorphous powder; [α]20 D +35.5 (*c* 0.1, MeOH); IR (KBr) ν_{max} (cm^{-1}) 3440, 2961, 1611, and 1509; UV λ_{max} (MeOH) 204 (0.78) and 263 (0.83) nm; negative ion HR-ESI-MS, *m/z* 573.1554 $[M+Cl]^-$ (calcd for $C_{29}H_{30}ClO_{10}$, 573.1533); ^1H NMR and ^{13}C NMR data: Tables 1 and 2.

2.3.3. Cremaphenanthrene N (*3*)

Brown amorphous powder; [α]20 D −27.9 (*c* 0.15, MeOH); IR (KBr) ν_{max} (cm^{-1}) 3399, 2924, and 1634; UV λ_{max} (MeOH) 204 (0.90) and 263 (0.97) nm; negative ion HR-ESI-MS *m/z* 573.1520 $[M+Cl]^-$ (calcd for $C_{29}H_{30}ClO_{10}$, 573.1533); ^1H NMR and ^{13}C NMR data: Tables 1 and 2.

2.3.4. Cremaphenanthrene O (*4*)

Brown amorphous powder; [α]20 D −11.5 (*c* 0.12, MeOH); IR (KBr) ν_{max} (cm^{-1}) 3392, 2920, 1613, 1460, and 1033; UV λ_{max} (MeOH) 204(0.97) and 264 (0.90) nm; positive ion HR-ESI-MS *m/z* 577.1703 $[M+Na]^+$ (calcd for $C_{29}H_{30}O_{11}Na$, 577.1686); ^1H NMR and ^{13}C NMR data: Tables 1 and 2.

2.3.5. Cremaphenanthrene P (*5*)

Brown amorphous powder; [α]20 D −14.3 (*c* 0.07, MeOH); IR (KBr) ν_{max} (cm^{-1}) 3452, 2965, 1509, and 1236; UV λ_{max} (MeOH) 204 (1.17), 262 (0.93), and 285 (0.74) nm; positive ion HR-ESI-MS *m/z* 555.1870 $[M+H]^+$ (calcd for $C_{29}H_{31}O_{11}$, 555.1866); ^1H NMR and ^{13}C NMR data: Tables 1 and 2.

Table 1
^{1}H NMR data of compounds **1**–**5**.

Position	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[b]
3	6.96 s	6.97 s	7.33 s	6.93 s	6.93 s
5	9.38 d (9.5)	9.41 d (9.5)	9.45 d (9.5)	9.19 d (9.6)	8.90 d (9.6)
6	7.18 dd (9.5, 3.0)	7.29 dd (9.5, 3.0)	7.09 dd (9.5, 3.0)	7.14 d (9.6)	7.43 d (9.6)
8	7.33 d (3.0)	7.44 d (3.0)	7.13 d (3.0)		
9	7.65 d (9.5)	7.62 d (9.5)	7.51 d (9.5)	8.32 d (9.6)	8.00 d (9.6)
10	7.81 d (9.5)	7.82 d (9.5)	7.82 d (9.5)	7.78 d (9.6)	7.77 d (9.6)
2′	6.86 d (2.5)	6.86 d (2.5)	6.80 d (2.5)	6.85 d (1.8)	6.85 d (1.8)
5′	6.57 d (8.5)	6.57 d (8.5)	6.62 d (8.5)	6.55 d (7.8)	6.55 d (7.8)
6′	6.46 dd (8.5,2.5)	6.46 dd (8.5, 2.5)	6.61 dd (8.5, 2.5)	6.43 dd (7.8, 1.8)	6.44 dd (7.8, 1.8)
7′	4.24 s	4.24 s	4.39 d (16.0)	4.18 d (15.6)	4.22 s
			4.54 d (16.0)	4.25 d (15.6)	
4-OCH_3	4.04 s	4.05 s	4.14 s	4.02 s	4.01 s
7-OCH_3	3.89 s				
3′-OCH_3	3.67 s	3.68 s	3.71 s	3.67 s	3.67 s
2-OH	9.68 s				
4′-OH	8.56 s				
1″		5.02 d (7.0)	5.04 d (7.0)	4.60 d (7.8)	4.70 d (7.8)
2″		3.31–3.38 m[c]	3.54–3.58 m	3.43–3.46 m	3.33–3.38[f]
3″		3.31–3.40 m[c]	3.50–3.54 m[d]	3.24–3.29 m[e]	3.26–3.33
4″		3.20–3.22 m	3.36–3.41 m	3.23–3.27 m[e]	3.18–3.20 m
5″		3.39–3.41 m	3.50–3.54 m[d]	3.16–3.20 m	3.33–3.37[f]
6″		3.73 br d	3.94 br d	3.62 br d	3.76 br d
		3.48–3.51 m	3.68–3.72 m	3.49–3.52 m	3.50–3.52 m

[c,d,e,f]Overlapped signal.
[a] ^{1}H NMR data were measured at 500 MHz in DMSO-d_6 for **1**–**2**, in CD_3OD for **3**.
[b] ^{1}H NMR data were measured at 600 MHz in DMSO-d_6 for **4**–**5**.

*2.4. Complete acid hydrolysis and GC analysis of compounds **2**–**5***

Compound **2**–**5** (1.0 mg, respectively) was stirred at 100 °C for 8 h with 2 M CF_3COOH (EtOH:H_2O, 1:1, 2 mL). The solution was cooled to room temperature and concentrated under reduced pressure. The residue was then redissolved in 2 mL pyridine with 0.1 M L-cysteine methyl ester hydrochloride and stirred at 60 °C for 1.5 h. After being cooled and concentrated, the mixture was treated with trimethylchlorosilane and hexamethyl-disilazane (1:2 v/v, 0.5 mL) in pyridine (2 mL), followed by stirring at 60 °C for 0.5 h. Then, the solution was concentrated to dryness and added with H_2O (2 × 3 mL), followed by extraction with *n*-hexane (2 × 3 mL) [6]. The *n*-hexane layer was analyzed by GC using a DB-5 column (0.25 mm × 30 m). The temperature of both the injector and detector was 220 °C. A temperature gradient system was applied to the oven, starting at 140 °C for 1 min and increasing up to 250 °C at a rate of 4 °C/min. Peaks of the sugar derivatives were identified by comparison with retention times to authentic sample of D-glucose (19.28 min) treated as above.

2.5. Cytotoxic bioassay

Cytotoxic activity was evaluated by MTT assay as previously reported [2]. Paclitaxel was served as positive control. Data are presented as mean for three independent experiments. Each concentration of compounds was tested in three parallel wells. IC_{50} values were calculated using Microsoft Excel software.

3. Results and discussion

Compound **1** (Tables 1 and 2, Fig. 2) was obtained as a brown amorphous powder. The negative ion HR-ESI-MS showed an ion at *m/z* 389.1398 $[M-H]^-$ and established the molecular formula as $C_{24}H_{22}O_5$. The IR spectrum showed absorption bands for hydroxy (3356 cm^{-1}), methylene

Table 2
^{13}C NMR data of compounds **1**–**5**.

Carbon	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[b]
1	113.2	113.3	118.9	113.1	113.2
2	153.0	153.2	154.7	153.0	153.4
3	99.5	99.5	100.9	99.5	99.4
4	156.9	156.9	159.3	157.0	157.3
5	128.8	128.7	131.1	125.1	118.6
6	116.4	116.9	117.6	117.2	116.9
7	156.0	154.1	156.0	145.4	140.3
8	108.4	112.3	112.2	138.8	140.9
9	127.4	127.4	128.7	121.5	120.9
10	123.6	123.5	124.9	123.3	122.2
4a	114.2	114.0	118.9	114.0	114.0
4b	124.6	125.4	125.6	124.3	126.8
8a	132.0	131.8	135.0	126.4	121.2
10a	132.5	132.5	133.8	132.2	133.0
1′	132.5	132.7	135.0	132.5	132.5
2′	112.7	112.7	113.4	112.6	112.6
3′	147.3	147.3	149.0	147.3	147.3
4′	144.3	144.3	145.4	144.3	144.3
5′	115.2	115.2	116.0	115.2	115.2
6′	120.1	120.1	121.9	120.1	120.1
7′	29.5	29.5	31.4	29.5	29.6
4-OCH_3	55.8	55.6	56.5	55.5	55.5
7-OCH_3	55.0				
3′-OCH_3	55.5	55.5	56.4	55.6	55.6
1″		100.5	104.1	106.3	103.6
2″		73.3	75.3	74.1	73.5
3″		76.7	78.5	76.1	75.7
4″		69.7	71.9	69.5	69.9
5″		77.1	78.7	77.2	77.4
6″		60.7	63.0	60.6	60.4

[a] ^{13}C NMR data were measured at 125 MHz in DMSO-d_6 for **1**–**2**, in CD_3OD for **3**.
[b] ^{13}C NMR data were measured at 150 MHz in DMSO-d_6 for **4**–**5**.

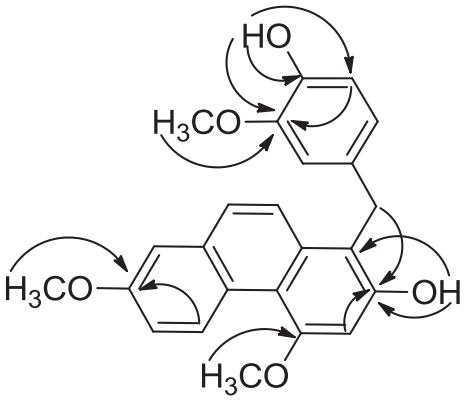

Fig. 2. Key HMBC correlations of compound **1**.

(2934 cm^{-1}), and aromatic functional groups (1611, 1514, 1454, 1368, 1277, and 1230 cm^{-1}), respectively [7]. The UV spectrum showed absorption maxima at 203 and 264 nm. The ^1H NMR spectrum displayed two exchangeable phenolic hydroxy singlet signals at δ_H 9.68 (1H, s, 2-OH) and 8.56 (1H, s, 4′-OH); nine aromatic proton signals consisting of one singlet signal at δ_H 6.96 (1H, s, H-3), a pairs of doublet signals at δ_H 7.65 (1H, d, J = 9.5 Hz, H-9) and 7.81 (1H, d, J = 9.5 Hz, H-10), two sets of ABX coupling systems at δ_H 9.38 (1H, d, J = 9.5 Hz, H-5), 7.18 (1H, dd, J = 9.5, 3.0 Hz, H-6), 7.33 (1H, d, J = 3.0 Hz, H-8), 6.86 (1H, d, J = 2.5 Hz, H-2′), 6.57 (1H, d, J = 8.5 Hz, H-5′), and 6.46 (1H, dd, J = 8.5, 2.5 Hz, H-6′); three methoxy singlet signals at δ_H 4.04 (3H, s, H-4-OCH_3), 3.89 (3H, s, H-7-OCH_3) and 3.67 (3H, s, H-3′-OCH_3); and a benzylic methylene singlet signal at δ_H 4.24 (2H, s, H-7′). In the ^{13}C NMR spectrum, twenty aromatic, one methylene, and three methoxy carbon signals were observed. This information, especially the presence of the deshielded proton signals δ_H 9.38 (H-5), indicated the characteristics of benzylphenanthrene for compound **1**. The 1D NMR data were similar to those of 1-(3′-methoxy-4′-hydroxybenzyl)-4-methoxyphenanthrene-2,7-diol [4] except that a methoxyl replaced the hydroxyl at C-7. This was further confirmed by HMBC correlations between H-5 and C-7, H-7-OCH_3 and C-7. Therefore, the structure of **1** was established as 1-(3′-methoxy-4′-hydroxybenzyl)-4,7-dimethoxyphenanthrene-2-ol, and named as cremaphenanthrene L.

Compound **2** (Tables 1 and 2, Fig. 3) was present to be a brown amorphous powder, with the molecular formula of $C_{29}H_{30}O_{10}$ determined from the negative ion HR-ESI-MS at m/z 573.1554 $[M+Cl]^-$. In the ^{13}C NMR spectrum, six carbon signals (δ_C 100.5, 73.3, 76.7, 69.7, 77.1, and 60.7) assigned to a glucopyranosyl moiety were observed. The coupling constant of the anomeric proton (7.0) found in ^1H NMR spectrum indicated it to be a β-glc, acid hydrolysis of **2** gave D-glucose, confirmed by comparison with an authentic sample. Meanwhile, twenty aromatic, one methylene, and two methoxy carbon signals in the ^{13}C NMR spectrum suggested the presence of a benzylphenanthrene moiety, whose NMR spectroscopic data were quite similar to those of 1-(3′-methoxy-4′-hydroxybenzyl)-4-methoxyphenanthrene-2,7-diol [4], the existence of the same benzylphenanthrene moiety was further supported by the HSQC, HMBC, and NOESY experiments. The glucopyranosyl moiety was determined to be located at C-7 based on the HMBC correlation between H-1″ (δ_H 5.02) and C-7 (δ_C 154.1) and the NOESY correlations between H-1″ (δ_H 5.02) and H-6 (δ_H 7.29), H-8 (δ_H 7.44). Thus, the structure of **2** was elucidated as 1-(3′-methoxy-4′-hydroxybenzyl)-4-methoxyphenanthrene-2-ol-7-*O*-β-D-glucopyranoside, and named as cremaphenanthrene M.

Compound **3** (Tables 1 and 2, Fig. 3) was isolated as a brown amorphous powder. The molecular formula of **3** was determined as $C_{29}H_{30}O_{10}$ on the basis of the negative ion HR-ESI-MS at m/z 573.1520 $[M+Cl]^-$. The same molecular formula and the highly similar IR, UV, 1D NMR spectra of **3** with those of **2** indicated that **3** was also a benzylphenanthrene glycoside. The main difference was the linkage position of glucopyranosyl moiety. The HMBC correlations between H-1″ (δ_H 5.02) and C-2 (δ_C 154.7) as well as the NOESY correlations between H-1″ (δ_H 5.02) and H-3 (δ_H 7.33) confirmed that the glucopyranosyl moiety was connected to C-2. The β-D-glc unit was further deduced by the coupling constant of the anomeric proton (7.0) and acid hydrolysis. Accordingly, we declared the structure of **2** as 1-(3′-methoxy-4′-hydroxybenzyl)-4-methoxyphenanthrene-7-ol-2-*O*-β-D-glucopyranoside, and named as cremaphenanthrene N.

Compound **4** (Tables 1 and 2, Fig. 4) was also obtained as a brown amorphous powder. A positive ion at m/z 577.1703

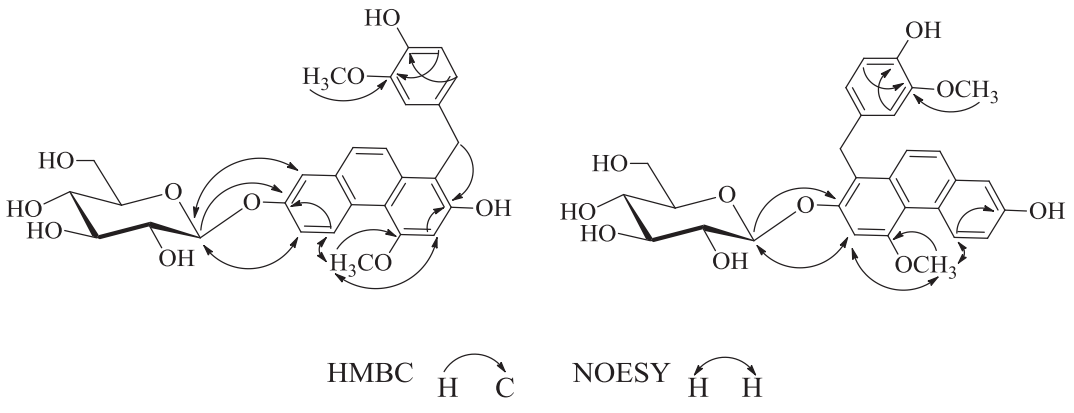

Fig. 3. Key HMBC and NOESY correlations of compounds **2**–**3**.

$[M+Na]^+$ was found in the HR-ESI-MS spectrum of **4**, indicating the molecular formula of $C_{29}H_{30}O_{11}$. The IR and UV spectra of **4** were similar to those of **3**. Six carbon signals (δ_C 106.3, 74.1, 76.1, 69.5, 77.2, and 60.6) assignable to a glucopyranosyl moiety were also observed in the ^{13}C NMR spectrum. Acid hydrolysis of **4** yielded D-glucose, identified by comparison with an authentic sample. In the ^{13}C NMR spectrum, twenty aromatic, one methylene and two methoxy carbon signals were observed. All the above data indicated **4** to be a benzylphenanthrene glycoside. The ^1H NMR spectrum displayed eight aromatic proton signals including one set of ABX coupling systems at δ_H 6.85 (1H, d, J = 1.8 Hz, H-2′), 6.55 (1H, d, J = 7.8 Hz, H-5′), and 6.43 (1H, dd, J = 7.8, 1.8 Hz, H-6′); one singlet signal at δ_H 6.93 (1H, s, H-3); two pairs of doublet signals at δ_H 8.32 (1H, d, J = 9.6 Hz, H-9), 7.78 (1H, d, J = 9.6 Hz, H-10), 9.19 (1H, d, J = 9.6 Hz, H-5) and 7.14 (1H, d, J = 9.6 Hz, H-6). Two methoxy singlet signals at δ_H 4.02 (3H, s, H-4-OCH_3) and 3.67 (3H, s, H-3′-OCH_3) were also observed in ^1H NMR spectrum of **4**. The substituent positions of benzylphenanthrene moiety were deduced by 2D-NMR experiments. The HMBC correlations from H-3 to C-1, C-2, C-4, and C-4a, H-5 to C-4a, C-7, and C-8a, H-9 to C-4b, C-8, C-8a and C-10a, H-10 to C-1, C-4a, C-8a and C-10a, H-4-OCH_3 to C-4, H-2′ to C-1′, C-3′, C-4′, C-6′ and C-7′, H-5′ to C-1′, C-3′, C-4′ and C-6′, H-6′ to C-2′, C-4′, C-5′ and C-7′, H-7′ to C-1′, C-2′, C-6′ and C-2, H-3′-OCH_3 to C-3′, The NOESY correlations between H-4-OCH_3 (δ_H 4.02) and H-3 (δ_H 6.93) and H-5 (δ_H 9.19), H-3′-OCH_3 (δ_H 3.67) and H-2′ (δ_H 6.85), these data showed the benzylphenanthrene moiety to be 1-(3′-methoxy-4′-hydroxybenzyl)-4-methoxyphenanthrene-2,7,8-triol. The glucopyranosyl moiety was determined to connect at C-8 on the basis of the HMBC correlation between H-1″ (δ_H 4.60) and C-8′ (δ_C 138.8) as well as the NOESY correlation between H-1″ (δ_H 4.60) and H-9 (δ_H 8.32). Subsequently, the structure of **4** was established as 1-(3′-methoxy-4′-hydroxybenzyl)-4-methoxyphenanthrene-2,7-diol-8-*O*-β-D-glucopyranoside, and named as cremaphenanthrene O.

Compound **5** (Tables 1 and 2, Fig. 4) was isolated as a brown amorphous powder. The molecular formula of **5** was determined as $C_{29}H_{30}O_{11}$ from the positive ion HR-ESI-MS at m/z 555.1870 $[M+H]^+$. The molecular formula is identical to that of **4**, the IR, UV, and 1D-NMR spectra of **5** were similar to those of **4**, which suggested that **5** was a benzylphenanthrene glycoside. The presence of D-glucose was elucidated by GC analysis of its acid hydrolysis derivation. The glucopyranosyl moiety linkage was determined to be C-7 due to the HMBC correlation between H-1″ (δ_H 4.70) and C-7 (δ_C 140.3) as well as the NOESY correlation between H-1″ (δ_H 4.70) and H-6 (δ_H 7.43). Eventually, we established the structure of **5** as 1-(3′-methoxy-4′-hydroxybenzyl)-4-methoxyphenanthrene-2,8-diol-7-*O*-β-D-glucopyranoside, and named as cremaphenanthrene P.

Table 3
Cytotoxicities of compounds **1** against tumor cell lines, IC_{50} value (μM).

Compounds	HCT-116	Hela	MCF-7	MDA-MB-231
1	19.01	68.81	24.18	15.84
Paclitaxel	2.33	0.08	0.52	0.002

The cytotoxicities of compounds **1**–**5** were evaluated by MTT Method, using paclitaxel as a positive control. Their cytotoxicities against colon (HCT-116), cervix (Hela), and breast (MCF-7, MDA-MB-231) cell lines were determined. The results (Table 3) indicated that **1** showed moderate cytotoxic activities against HCT-116, MCF-7 and MDA-MB-231 cancer cell lines with IC_{50} values ranging from 15.84 to 24.18 μM, and weak cytotoxicity to Hela cell line with IC_{50} value of 68.81 μM, while compounds **2**–**5** were inactive (IC_{50} > 100 μM).

Phenanthrenes have been reported mainly from the Orchidaceae family [8]. Our findings afford further evidences that phenanthrenes can serve as the taxonomic marker of this family [8]. Benzylphenanthrenes have been previously reported mainly from three genera of the Orchidaceae family: *Bletilla*, *Gymnadenia*, and *Spiranthes*, all of which belong to the Orchidoideae subfamily [8,9]. The occurrence of benzylphenanthrenes in *C. appendiculata* suggested that the *Cremastra* genus could have a close genetic relationship with the genera *Bletilla*, *Gymnadenia*, and *Spiranthes*.

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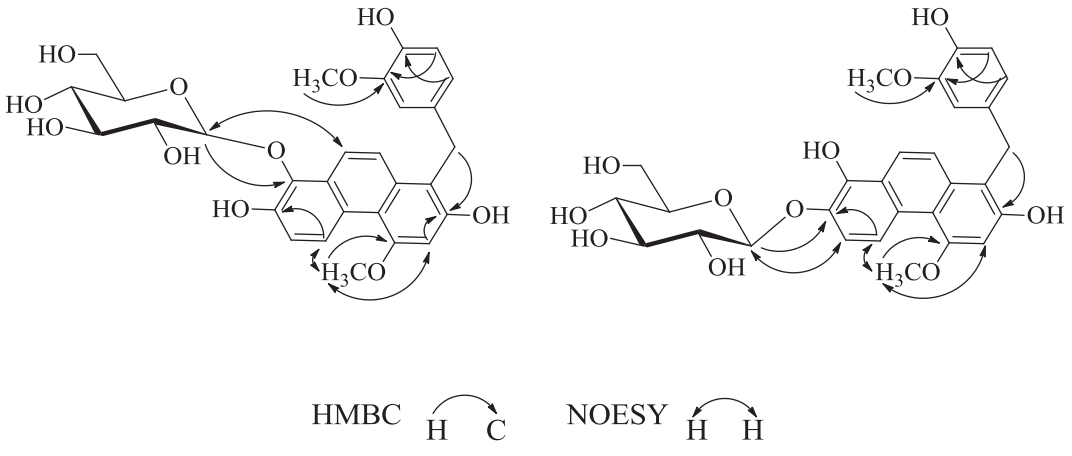

Fig. 4. Key HMBC and NOESY correlations of compounds **4**–**5**.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2015.03.003.

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