New Polyphenols and Triterpene from the Pseudobulbs of *Pleione formosana*

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Three new polyphenolic compounds including a dihydrophenanthrene, pleioanthrenin (**1**), two bibenzyls, pleiobibenzynin A (**2**) and B (**3**), and a new cycloartane triterpenoid, (24*R*)-cyclomargenyl *p*-coumarate (**4**), together with eight known compounds, were isolated from the pseudobulbs of *Pleione formosana*. The structures of the new compounds were elucidated by extensively spectroscopic analysis.

Keywords: *Pleione formosana*; Pseudobulbs; Orchidaceae; Dihydrophenanthrenes; Bibenzyls; Triterpenoids.

INTRODUCTION

Pleione formosana Hay. (Orchidaceae) is a perennial herb with rhizomes, corms, or root-stem tuberoids. The pseudobulbs of *P. formosana* have been used as one of the substitute of *Shan-ci-gu*, which is used in traditional Chinese medicine to treat tumors.[1,2] To the best of our knowledge, there has been no phytochemical investigation on *P. formosana*. As a part of our effort to search for bioactive components from local indigenous herbs, we describe herein the first isolation of a dihydrophenanthrene (**1**), two bibenzyls (**2** and **3**), and a cycloartane triterpenoid (**4**), together with 8 known compounds from the pseudobulbs of *P. formosana.* Their structures were established mainly by 1D and 2D NMR and mass spectroscopic analysis.

RESULTS AND DISCUSSION

The ethanolic extract of the pseudobulbs of *P. formosana* was successively partitioned with EtOAc and *n*-BuOH. The EtOAc and *n*-BuOH-soluble fractions were separatedly subjected to silica gel column chromatography (CC). Fractions rich in polyphenolic compounds were further separated and purified on Sephadex LH-20 column followed by C_{18} CC and semipreparative RP-18 HPLC to give a new dihydrophenanthrene, pleioanthrenin (**1**), two bibenzyls, pleiobibenzynin A (**2**) and B (**3**), and a cycloartane triterpenoid, (24*R*)-cyclomargenyl *p*-coumarate (**4**), together with 1-(4-hydroxybenzyl)-4,7-dimethoxy-9,10-dihydrophenanthrene-2-ol (**5**),[3] bulbocol (**6**),[4] 3′,5-dihydroxy-2-(4-hydroxylbenzyl)-3-methoxybibenzyl (**7**),[5] bulbocodin C (**8**),[6] arundin (**9**),[7] 3,3′-dihydroxy-2,6-bis(4-hydroxybenzyl)-5-methoxybibenzyl (**10**),[8] 2,6-bis(4-hydroxybenzyl)-3′,5-dimethoxy-3-hydroxybibenzyl (**11**),[8] (24*R*)-cyclomargenol (**12**).[9]

Compound **1** was isolated as a pale yellow amorphous powder with a molecular formula $C_{23}H_{22}O_4$ by HR-EI-MS. The IR spectrum showed absorptions at 3374 (OH), 1598 and 1461 (benzenoid) cm^{-1}. The UV absorption maxima at 269sh, 278, 297sh, and 317sh nm were characteristic of a dihydrophenanthrene skeleton.[10] The ^{1}H NMR spectrum of **1** (see Table 1) showed two methoxyl groups at δ 3.82 and 3.88 (3H each, s), an isolated aromatic proton at δ 6.64 (s, H-3), an ABX system aromatic protons at δ 8.00 (d, J = 8.0 Hz, H-5), 6.60 (dd, J = 8.0, 2.0 Hz, H-6) and 6.62 (d, J = 2.0 Hz, H-8), a *p*-substituted benzyl [δ 6.63 and 6.88 (2H each, d, J = 8.0 Hz), and 3.94 (2H, s)], and signals assignable to the 9- and 10-methylene protons [δ 2.58 and 2.50 (2H each, m) of dihydrophenanthrene. The NOE correlations (Fig. 1) of methoxyl at δ 3.82 to H-6 and H-8, and methoxyl at δ 3.88 to H-3′(5′) in the NOESY experiment of **1** revealed that the methoxyl functionality are attached to C-7 and C-4′, respectively. The assignments were further confirmed by HMBC experiment (Fig. 1) from methoxyl (δ 3.82) to C-7, from H-5 to C-6, C-4b, C-7, and C-8a, from H-7′ to C-1, C-1′, C-2′(6′), C-2, C-10a; from methoxyl (δ 3.88) to C-4′, from H-3′(5′) to C-1′, C-2′(6′), and C-4′ (Fig. 1). Comparison of the ^{1}H- and ^{13}C-NMR data of **1** are similar to those of 1-(4-hydroxybenzyl)-7-methoxy-9,10-dihydrophenanthrene-2,7-diol[3] except for a methoxyl group at

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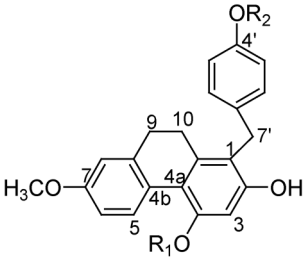
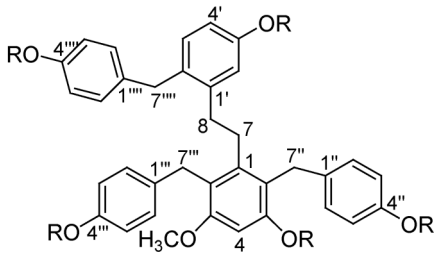
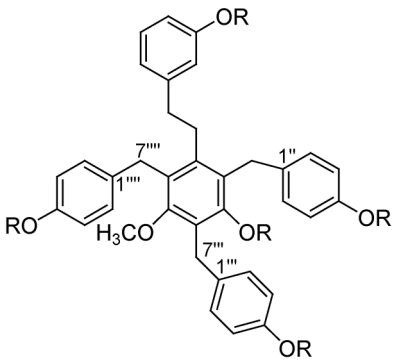


1 R_1=H, R_2=CH_3

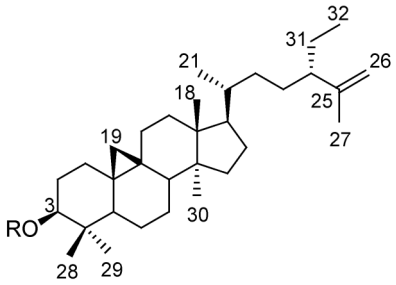
5 R_1=CH_3, R_2=H



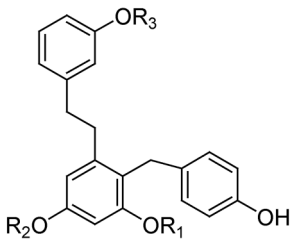
2 R=H
2a R=Ac



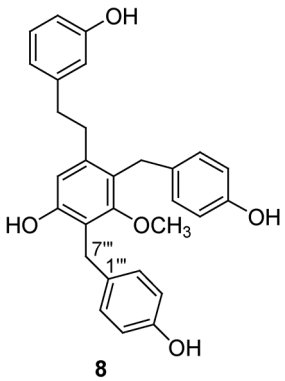
3 R=H
3a R=Ac



4 R= *trans*-coumaroyl
12 R=H



6 R_1, R_3=CH_3, R_2 =H
7 R_1=CH_3, R_2, R_3=H



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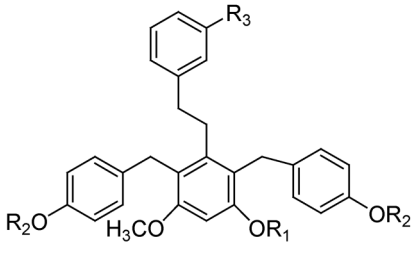

9 R_1, R_2, R_3=H
10 R_1, R_2=H, R_3=OH
11 R_1, R_2=H, R_3=OCH_3

4′-position in place of a hydroxyl. From above results, the structure of compound **1** was established as 1-(4-methoxybenzyl)-7-methoxy-9,10-dihydrophenanthrene-2,4-diol, named pleioanthrenin.

Compound **2** had a pseudomolecular ion at *m/z* 561.2246 $(M-H)^-$ with the molecular formula of $C_{36}H_{33}O_6$ as established by HR-ESI-MS. The UV absorption maxima at 225 sh and 283 nm indicated the presence of bibenzyl.[7] The IR spectrum showed absorption bands for hydroxyl (3362 cm^{-1}) and benzene ring (1600, 1513 and 1446 cm^{-1}). Acetylation of **2** afforded pentaacetate [δ_H 2.11, 2.24, 2.24, 2.26, and 2.27 (3H each, s)], suggesting the presence of five hydroxyl groups. The ^{1}H NMR spectrum of **2** (see Table 1) presented the signals of three *p*-hydroxybenzyl groups [δ 3.57 (2H, s), δ 6.58 and 6.76 (2H each, d, J = 8.0 Hz); δ 3.80 (2H, s), δ 6.59 and 6.76 (2H each, d, J = 8.0 Hz); δ 3.84 (2H, s), δ 6.63 and 6.85 (2H each, d, J = 8.0 Hz)], an ABX-system 1,3,6-trisubstituted phenyl group [δ 6.51 dd (J = 8.5, 2.0 Hz), δ 6.57 d (J = 2.0 Hz), 6.77 d (J = 8.5 Hz)], four aliphatic bibenzyl methylene protons [δ 2.42

Table 1. ^{1}H- and ^{13}C-NMR spectral data for compounds **1**~**3** in CD_3OD (δ, ppm)

Position	**1**		**2**		**3**	
	$^{1}H^{a}$	$^{13}C^{b}$	$^{1}H^{a}$	$^{13}C^{b}$	$^{1}H^{a}$	$^{13}C^{b}$
1		118.3 s		142.8 s		140.8 s
2		156.0 s		119.4 s		124.9 s
3	6.64 s	96.1 d		156.3 s		154.1 s
4		156.3 s	6.46 s	98.2 d		121.1 s
4a		120.1 s				
4b		126.1 s				
5	8.00 d (8.0)[c]	130.5 d		158.4 s		157.7 s
6	6.60 dd (8.0,2.0)	113.6 d		120.2 s		125.7 s
7		157.4 s	2.66 m	31.7 t	2.67 m	33.4 t
8	6.62 d (2.0)	114.6 d	2.42 m	34.6 t	2.35 m	37.7 t
8a		140.6 s				
9	2.58 m	30.9 t				
10	2.50 m	27.5 t				
10a		140.7 s				
1′		133.6 s		142.7 s		145.1 s
2′	6.88 d (8.0)	129.9 d	6.57 d (2.0)	116.5 d	6.47 dd (2.1,2.0)	116.0 d
3′	6.63 d (8.0)	115.9 d		156.6 s		158.3 s
4′		158.1 s	6.51 dd (8.5,2.0)	113.8 d	6.55 ddd (8.0,2.1,2.0)	113.7 d
5′	6.63 d (8.0)	115.9 d	6.77 d (8.5)	132.0 d	7.00 t (8.0)	130.1 d
6′	6.88 d (8.0)	129.9 d		131.4 s	6.46 ddd (8.0,2.1,2.0)	120.6 d
7′	3.94 s	30.9 t				
1″				134.2 s		133.4 s
2″			6.85 d (8.0)	129.9 d	6.90 d (8.2)	130.0 d
3″			6.63 d (8.0)	115.9 d	6.65 d (8.2)	115.9 d
4″				155.9 s		156.2 s
5″			6.63 d (8.0)	115.9 d	6.65 d (8.2)	115.9 d
6″			6.85 d (8.0)	129.9 d	6.90 d (8.2)	130.0 d
7″			3.84 s	31.1 t	4.03 s	32.0 t
1‴				134.1 s		133.4 s
2‴			6.77 d (9.0)	129.8 d	7.02 d (8.0)	130.2 d
3‴			6.58 d (9.0)	115.8 d	6.66 d (8.0)	116.0 d
4‴				155.9 s		156.2 s
5‴			6.59 d (9.0)	115.8 d	6.66 d (8.0)	116.0 d
6‴			6.76 d (9.0)	129.8 d	7.02 d (8.0)	130.2 d
7‴			3.80 s	31.0 t	4.03 s	30.1 t
1⁗				133.6 s		134.2 s
2⁗			6.76 d (8.0)	130.6 d	6.85 d (8.4)	129.9 d
3⁗			6.58 d (8.0)	116.1 d	6.63 d (8.4)	115.9 d
4⁗				155.9 s		156.2 s
5⁗			6.58 d (8.0)	116.1 d	6.63 d (8.4)	115.9 d
6⁗			6.76 d (8.0)	130.6 d	6.85 d (8.4)	129.9 d
7⁗			3.57 s	37.9 t	3.94 s	31.9 t
OMe	3.82 s	56.2 q	3.72 s	55.9 q	3.46 s	62.2 q
	3.88 s	56.3 q				

Spectra recorded at [a] 500 MHz and [b] 125 MHz in CD_3OD at 25 °C. [c] Coupling constants are presented in Hz.

and 2.66 (each 2H, m)], along with a methoxyl group [δ 3.72 (3H, s)]. The data was also supported by the ^{13}C NMR and DEPT experiment, which was analyzed by ^{1}H-^{13}C shim correlations by HMQC experiment. The location of hydroxyl, methoxyl and *p*-hydoxybenzyl were determined by 1D NOE, NOESY (Fig. 2) and HMBC (Fig. 2). Irradiation of the methoxyl at δ 3.72 caused the NOE enhancement of H-4 (δ 6.46 s), H-7‴ (δ 3.80 s) and H-2‴(6‴) (δ 6.76 d), irradiation of H-8 (δ 2.42 m) resulted the NOE with H-7⁗ (δ 3.57 s) and H-2′ [δ 6.57 (d, J = 2.0 Hz)], and irradiation of

H-7 (δ 2.66 m) caused the NOE enhancements of H-7″ (δ 3.84 s) and H-7‴ (δ 3.80 s) in 1D NOE experiments indicating the symmetrical substituted the *p*-hydroxybenzyls on C-2 and C-6, the other *p*-hydroxybenzyl on C-6′, and the methoxyl functionality attached to C-5, respectively. The HMBC spectrum revealed significant correlations between methoxyl (δ_H 3.72 s) and carbons at δ_C 158.4 (C-5), between H-8 (δ_H 2.42 m) and carbons at δ_C 31.7 (C-7), 116.5 (C-2′), 131.4 (C-6′), 142.7 (C-1′), and 142.8 (C-1), between H-7 (δ_H 2.66 m) and carbons at δ_C 34.6 (C-8), 119.4 (C-2), 120.2 (C-6), and 142.7 (C-1′), and 142.8 (C-1), between H-7″ (δ_H 3.84 s) and carbons at δ_C 119.4 (C-2), 129.9 (C-2″(6″)), 134.2 (C-1″), 142.8 (C-1), and 156.3 (C-3), between H-7‴ (δ_H 3.80 s) and carbons at δ_C 120.2 (C-6), 129.8 (C-2‴(6‴)), 134.1 (C-1‴), 142.8 (C-1), and 158.4 (C-5), between H-7⁗ (δ_H 3.57 s) and carbons at δ_C 130.6 [C-2⁗(6⁗)], 131.4 (C-6′), 132.0 (C-5′), 142.7 (C-1′), and 133.6 (C-1⁗), between H-4 (δ_H 6.46 s) and carbons at δ_C 119.4 (C-2), 120.2 (C-6), 156.3 (C-3), 158.4 (C-5), and between H-4′ [δ_H 6.51 (8.5, 2.0 Hz)] and carbons at δ_C 116.5 (C-2′), 132.0 (C-5′), 131.4 (C-6′), and 156.6 (C-3′). From above results, the structure of **2** was assigned as 3,3′-dihydroxy-5-methoxy-2,6,6′-tri(*p*-hydroxybenzyl)bibenzyl, named pleiobibenzynin A.

Pleiobibenzynin B (**3**) has the same molecular formula with compound **2** as determined by negative HR-ESI-MS. The IR and UV spectra of **3** were similar to those of **2**. The ^{1}H, ^{13}C and DEPT spectra of **3** presented three *p*-hydroxybenzyl, four bibenzyl methylene protons, a methoxyl, and a system of 1,3-substituted phenyl protons. Pentaacetate [δ_H 2.08, 2.25, 2.25, 2.26, and 2.27 (3H each, s)] from its acetylation suggested the presence of five hydroxyl groups. The difference between compounds **2** and **3** was the position of one of the *p*-hydroxybenzyl at C-6′ in **2** instead of C-4 in **3**. These results were confirmed by selected NOE irradiations. The key NOE correlations in a NOESY experiment and HMBC correlations were shown in Fig. 3. The structure of the new compound was thus assigned as 3,3′-dihydroxy-5-methoxy-2,4,6-tri-(*p*-hydroxybenzyl)-bibenzyl.

Compound **4** was obtained as colorless powder, $[\alpha]_D^{20}$ +58° (*c* = 0.2, MeOH). A molecular formula of $C_{41}H_{59}O_3$ was determined by negative HR-ESI-MS (*m/z* 599.4454 $[M-H]^-$) with 12 degrees of unsaturation. The IR spectra displayed the presence of hydroxyl (3376 cm^{-1}) and an α,β-unsaturated ester (1705 and 1257 cm^{-1}). The ^{13}C-NMR and DEPT showed 41 carbons including seven methyls, thirteen methylenes (one sp^2), twelve methines (one oxygenated, 6 sp^2), eight quaternary carbons (two sp^2 and one oxygenated sp^2), and one conjugated carbonyl. The ^{1}H NMR spectrum showed signals indicating two characteristic pair of high field doublet of cyclopropanyl methylene protons [δ 0.37 (1H, d, *J* = 4.0 Hz), 0.61 (1H, d, *J* = 4.0 H)], five tertiary methyls [δ 0.90, 0.91, 0.98, 0.98, 1.63 (3H each, s)], a secondary methyl [δ 0.87 (3H, d, *J* = 6.5 Hz)], a primary methyl [δ 0.82 (3H, t, *J* = 7.5 Hz)], an *exo*-methylenes [δ 4.65 and 4.74 (1H each, br s)], an oxygenated methine [δ 4.71 (1H, dd, *J* = 9.0, 8.0 Hz)], an AA′BB′ system of aromatic protons [δ 6.84 and 7.43 (2H each, d, *J* =

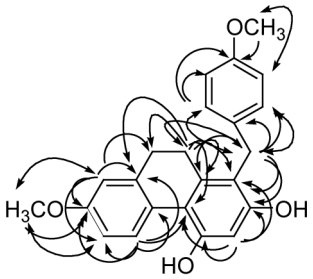

Fig. 1. Key NOE (↔) and HMBC (→) correlations observed for **1**.

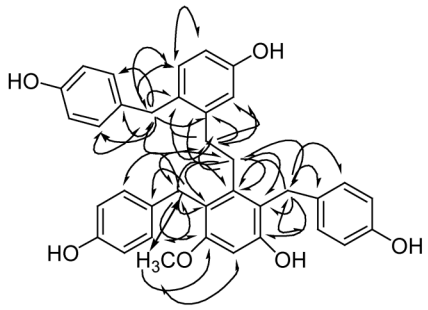

Fig. 2. Key NOE (↔) and HMBC (→) correlations observed for **2**.

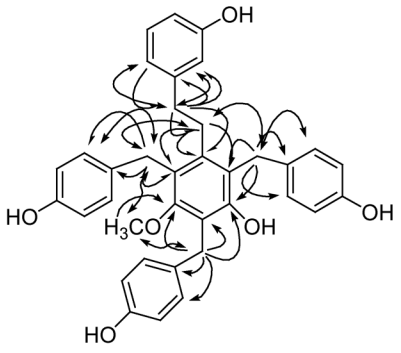

Fig. 3. Key NOE (↔) and HMBC (→) correlations observed for **3**.

8.5 Hz)], and an α,β-unsaturated olefinic protons [δ 6.31 and 7.61 (1H each, d, J = 16.0 Hz)]. The HMQC spectrum facilitated the assignment of the protonated carbons. From above data in addition to the coumaroyl moiety (with six degrees of unsaturation), the remaining six degrees of unsaturation suggested compound **4** to be a cycloartane-type triterpene like 24-ethyl-25-methylenecycloartanol.[9] The configuration of OH-3 must be in β-equatorial orientation, since H-3 had a bigger coupling constant [δ 4.71 (1H, dd, J = 9.0, 8.0 Hz)]. The assignment was further confirmed by the observation of main NOE correlation from H-3 and H-5 (δ_H 1.54 m), and key HMBC correlations between H-3 and carbonyl (δ_C 167.6), C-28 (δ_C 19.5), and C-29 (δ_C 15.6); between H-α [δ_H 6.31 (d, J = 16.0)] and C-β (δ_C 144.3), C-1′ (δ_C 127.6), and carbonyl (δ_C 167.6); between H-19 [δ_H 0.37 and 0.61 (1H each, d, J = 4.0 Hz)] and C-1 (δ_C 26.1), C-5 (δ_C 48.1), C-8 (δ_C 47.5), C-9 (δ_C 20.4), C-10 (δ_C 26.2), and C-11 (δ_C 26.8); between H-27 (δ_H 1.63 s) and C-24 (δ_C 49.8), C-25 (δ_C 147.8), and C-26 (δ_C 111.6); and between H-32 [δ_H 0.82 (t, J = 7.5 Hz) and C-24 and C-31 (δ_C 30.0)]. The absolute configuration at C-24 of **4** was accomplished by measuring the circular dichroism (CD) curve [$CD[\theta]_{283}$ -134.7°] and comparing with those of (24*R*)-cyclolaudenyl acetate and (24*R*)-cyclomargenyl acetate,[9] and concluded to have a (24*R*)-configuration. This is a first isolation from natural product.

EXPERIMENTAL SECTION

General Method

IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrophotometer. UV spectra were measured on a Hitachi U-3310 spectrophotometer. NMR spectra were run on a Varian unity INOVA-500 and Bruker AVANCE 400 spectrometers. Mass spectra (EI-MS, ESI-MS and HR-ESI-MS) were recorded on a Finnigan LCQ, Finnigan Mat-95 XL, respectively. Optical rotation was recorded on a Jasco DIP-370. Column chromatography were performed using Merck silica gel (kieselgel 60), Sephadex™ LH-20 (GE Healthcare) and RP-18 (Cosmosil 75C_{18}-PREP). Futher purifications were subjected by HPLC on RP-18 column (Merck, LiChrospher 100, RP-18e (5 μm), 250 × 10 mm). Si gel 60F_{254} (Merck) was used for TLC with 35% EA/hexane or 10% MeOH/$CHCl_3$ as developing solvent.

Plant Material

The pseudobulbs of *P. formosana* was purchased from Mei-Feng highland experimental farm of National Taiwan University, Ren-Ai Township, Nantou County, Aug in 2006. The plant was identified by Mr. Jun-Chih Ou, a previous associate research fellow of National Research Institute of Chinese Medicine.

Extraction and Isolation

Slices of pseudobulbs of *P. formosana* (3.2 kg) were extracted at 60 °C using 95% EtOH overnight (3 × 40 L). The EtOH extract (164 g) was taken up in H_2O, and partitioned with EtOAc (1:1, v/v) to give an EtOAc-soluble fraction (52.0 g) and aqueous phase. The aqueous phase was further extracted with *n*-BuOH to obtain *n*-BuOH-soluble (30.2 g) and H_2O-soluble (131.0 g) fractioncts. Both EtOAc and *n*-BuOH fractions were separatedly subjected to column chromatography over silica gel (using hexane-EtOAc-20% EtOAc/MeOH gradient for EtOAc extract, and 25% EtOAc/hexane ~ EtOAc-50% EtOAc/MeOH for *n*-BuOH extract). Fractions 50% EA/hexane ~ 50% EA/MeOH eluate rich in polyphenols were combined and re-chromatographed over Sephadex LH-20 column with MeOH as eluent to yield four fractions (II~IV). Fraction I was further purified over silica gel column with 20~35% EtOAc/hexane to give (24*R*)-cyclomargenyl *p*-coumarate (**4**) (72 mg) and (24*R*)-cyclomargenol (**12**) (296 mg). Fractions II~IV were repeatedly subjected to RP-18 (80% MeOH~MeOH) column, followed by semi-preparative HPLC using 80% MeOH/H_2O as mobile phase (2 mL/min) to give **1** (26 mg), **2** (15 mg), **3** (25 mg), **5** (21 mg), **6** (28 mg), **7** (25 mg), **8** (14 mg), **9** (38 mg), **10** (82 mg), and **11** (23 mg).

Pleioanthrenin (1)

Pale yellow amorphous powder; IR (KBr) υ_{max} 3374, 1605, 1596, 1509, 1461, 1239, 1081, 1042, 816 cm^{-1}; UV (MeOH) λ_{max} (log ε) 269sh (3.94), 278 (4.03), 297sh (3.93), 317sh (3.75) nm; ^1H NMR (500 MHz, methanol-d_4) and ^{13}C NMR: see Table 1; Main NOE and key HMBC correlations see Fig. 1; EIMS *m/z* 362 $(M)^+$ (100), 270 (50), 256 (25), 107 (18); HREIMS *m/z* 362.1513 (calcd for $C_{23}H_{22}O_4$ 362.1519).

Pleiobibenzynin A (2)

Colorless amorphous powder; IR (KBr) υ_{max} 3362, 1600, 1513, 1446, 1232, 1172, 1109, 1046, 816 cm^{-1}; UV (MeOH) λ_{max} nm (log ε): 225 sh (3.64), 283 (3.04) nm; ^1H NMR (500 MHz, methanol-d_4) and ^{13}C NMR: see Table 1; Main NOE and HMBC correlations: see Fig. 2; ESIMS negative mode *m/z* 561 $(M-H)^-$; negative-ion HRESIMS *m/z* 561.2246 (calcd for $C_{36}H_{33}O_6$ 561.2278).

Pleiobibenzynin B (3)

Colorless amorphous powder; IR (KBr) υ_{max} 3346, 1614, 1589, 1507, 1455, 1238, 1170, 1082, 1047, 816 cm^{-1}; UV (MeOH) λ_{max} (log ε) 225 sh (3.65), 283 (3.02) nm; ^1H NMR (500 MHz, methanol-d_4) and ^{13}C NMR see Table 1; Main NOE and HMBC correlations see Fig. 3; ESIMS negative mode *m/z* 561 $(M-H)^-$; negative-ion HRESIMS *m/z* 561.2222 (calcd for $C_{36}H_{33}O_6$, 561.2278).

(24*R*)-cyclomargenyl *p*-coumarate (4)

Colorless amorphous powder; $[\alpha]_D^{20}$ +58° (*c* = 0.2, MeOH); IR (KBr) υ_{max} 3376, 2928, 2867, 1705, 1601, 1585, 1513, 1445, 1374, 1257, 1167, 1025, 900, 830 cm^{-1}; ^1H NMR (500 MHz, chloroform-*d*): δ 0.37 and 0.61 (1H each, d, *J* = 4.0 Hz, H-19), 0.82 (3H, t, *J* = 7.5 Hz, H-32), 0.87 (3H, d, *J* = 6.5 Hz, H-21), 0.90 (3H, s, H-28), 0.91 (3H, s, H-30), 0.98 (3H, s, H-18), 0.98 (3H, s, H-29), and 1.63 (3H each, s, H-27), 4.65 and 4.74 (1H each, br s, H-26), 4.71 (1H, dd, *J* = 9.0, 8.0 Hz, H-3α), 5.62 (1H, br s, OH), 6.31 and 7.61 (1H each, d, *J* = 16.0 Hz, H-α, β), 6.84 and 7.43 (2H each, d, *J* = 8.5 Hz, H-3″(5″), 2″(6″)); ^{13}C NMR (125 MHz, chloroform-*d*): δ 12.3 q (C-32), 15.6 q (C-29), 18.0 q (C-27), 18.2 q (C-30), 18.5 q (C-21), 19.5 q (C-28), 20.4 s (C-9), 21.2 t (C-6), 25.7 q (C-18), 26.1 t (C-1), 26.2 s (C-10), 26.8 t (C-11), 26.8 t (C-16), 27.2 t (C-7), 28.3 t (C-19), 30.0 t (C-31), 30.1 t (C-23), 31.9 t (C-22), 33.1 t (C-12), 34.2 t (C-2), 35.8 t (C-15), 36.1 d (C-20), 39.9 s (C-13), 45.5 s (C-14), 47.5 d (C-8), 48.1 d (C-5), 49.0 s (C-4), 49.8 d (C-24), 52.5 d (C-17), 80.9 d (C-3), 111.6 t (C-26), 116.1 d (C-3′(5′)), 116.6 d (C-α), 127.6 s (C-1′), 130.2 d (C-2′(6′)), 144.3 d (C-β), 147.8 s (C-25), 157.8 s (C-4′), 167.6 s (C=O); Main NOE: H-3/H-5, H-29; H-18/H-16; H-19/H-1, H-11; and H-28/H-2; Key HMBC correlations: H-3/C=O, C-28, C-29; H-α/C-β, C-1′, C=O; H-19/C-1, C-5, C-9, C-10; H-21/C-17, C-20, C-22; H-26/C-24, C-25, C-27; H-27/C-24, C-25, C-26; H-30/C-8, C-13, C-14, C-15; and H-32/C-24 and C-31. ESIMS negative mode: 599 $[M-H]^-$; negative-ion HRESIMS *m/z* 599.4374 (calcd for $C_{41}H_{59}O_3$ 599.4467); $CD[\theta]_{283}$ -134.7°.

Acetylation of 2 and 3

Compound **2** or **3** (4 mg) was allowed to react with Ac_2O (1.0 mL) in pyridine (0.5 mL) at 60 °C for overnight, respectively. Usual work-up gave pentaacetate of **2** (**2a**): Colorless amorphous; ^1H NMR (500 MHz, chloroform-*d*) δ 2.11, 2.24, 2.24, 2.26, and 2.27 (3H each, s), 3.72, 3.77, and 3.97 (2H each, s), 3.73 (3H, s), 6.59 (1H, s), 6.69 (1H, d, *J* = 2.0 Hz), 6.82 (1H, dd, *J* = 8.0, 2.0 Hz), 6.89 (2H, d, *J* = 8.0 Hz), 6.90 (3H, d, *J* = 8.0 Hz), 6.91 (2H, d, *J* = 8.0 Hz), 6.93 (2H, d, *J* = 8.0 Hz), 6.94 (2H, d, *J* = 8.0 Hz), 6.96 (2H, d, *J* = 8.0 Hz), 6.99 (2H, d, *J* = 8.0 Hz); pentaacetate of **3** (**3a**): Colorless amorphous; ^1H NMR (500 MHz, chloroform-*d*) δ 2.08, 2.25, 2.25, 2.26, and 2.27 (3H each, s), 3.51 (3H, s), 3.93, 4.07, and 4.07 (2H each, s), 6.62 (1H, dd, *J* = 2.1, 2.0 Hz), 6.72 (1H, ddd, *J* = 8.0, 2.1, 2.0 Hz), 6.82 (1H, ddd, *J* = 8.0, 2.1, 2.0 Hz), 6.92 (2H, d, *J* = 8.5 Hz), 6.95 (2H, d, *J* = 8.5 Hz), 6.95 (2H, d, *J* = 8.5 Hz), 7.01 (2H, d, *J* = 8.5 Hz), 7.03 (2H, d, *J* = 8.5 Hz), 7.04 (1H, t, *J* = 8.0 Hz), 7.12 (2H, d, *J* = 8.5 Hz).

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