CHEMISTRY & BIODIVERSITY

Hypofolins A – L, *ent*-Labdane Diterpenoids from the Roots of *Hypoestes phyllostachya* 'Pink Splash'

Bin Cheng,[a,b] Lin-Fen Ding,[b] Tong Yan,[a,b] Zhang-Qiao Xie,[a,b] Zhi-jun Zhang,[a] Liu-Dong Song,[b] Xing-De Wu,*[a] and Qin-Shi Zhao*[a]

[a]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, P. R. China, e-mail: wuxingde@mail.kib.ac.cn, qinshizhao@mail.kib.ac.cn
[b]School of Pharmaceutical Science & Yunnan Key Laboratory of Pharmacology for Natural Products, Kunming Medical University, Kunming 650500, P. R. China

Twelve new *ent*-labdane diterpenoids, hypofolins A – F (**1** – **6**) and hypofolins G – L (**7a**/**7b**, **8a**/**8b**, and **9a**/**9b**), were isolated from the roots of *Hypoestes phyllostachya* 'Pink Splash'. Their structures were elucidated by extensive 1D- and 2D-NMR spectroscopic and HR-MS data. The absolute configurations of **1**, **2**, **5**, and **7a**/**7b** were determined by single crystal X-ray diffraction and ECD analysis, as well as chemical transformations. Compounds **7a**/**7b**, **8a**/**8b**, and **9a**/**9b** were isolated as three pairs of interconverting mixture of two isomers between ketone and hemiketal types. Compound **1** showed weak cytotoxicity against SMMC-7721 cell line with IC_{50} value of 31.40 μM.

Keywords: *Hypoestes phyllostachya*, *ent*-labdane diterpenoids, hypofolins A – L, cytotoxicity, NO production inhibitory activity.

Introduction

Plants of genus *Hypoestes* (Acanthaceae), comprising 40 species, are mainly distributed throughout the tropical and subtropical lands around the Indian Ocean, and some adjacent regions.[1 – 3] Species of the genus *Hypoestes* are used in fork medicine for the treatment of high blood pressure, cancer, infection vaginitis, and heart diseases, and as antipyretic and antiphlogistic agents.[4 – 6] Phytochemically, plants of the genus *Hypoestes* are rich sources of diterpenoids, which display a variety of bioactivities including anti-cancer, anti-inflammatory, antifungal, antiparasitic, and cytotoxic activities.[4 – 15] Especially, hypoestoxide, a novel verticillane diterpenoid isolated from *Hypoestes rosea*, has attracted much attention from biologists and has been reported to exhibit anti-inflammatory and anti-cancer activities.[7 – 9]

H. phyllostachya are an economically important horticultural plant, which has many different cultivars with ovate leaves marked with pink, white, or red spots.[16][17] Our previous chemical study on the aerial parts of *H. phyllostachya* 'Rosea' (one of cultivars of *H. phyllostachya*) led to the isolation of six labdane diterpenoids with potent vasorelaxant activity on endothelium-intact thoracic aorta rings precontracted with KCl.[17] In continuation of our research for structurally novel and biologically active constituents from this species, twelve new terpenoids, hypofolins A – F (**1** – **6**) and hypofolins G – L (**7a**/**7b**, **8a**/**8b**, and **9a**/**9b**), were isolated from the roots of *H. phyllostachya* 'Pink Splash'. Among them, compounds **5** and **6** were two rare norditerpenoids, and compounds **7a**/**7b**, **8a**/**8b**, and **9a**/**9b** were isolated as three pairs of interconverting mixture of two isomers between ketone and hemiketal types. All compounds were tested for their cytotoxicities against HL-60, SMMC-7721, A-549, MCF-7, and SW-480 cell lines and inhibitory activity against LPS-induced NO production in RAW264.7 macrophages. In the present article, we describe the isolation, structural elucidation of these compounds, and their biological evaluation (*Figure 1*).

Results and Discussion

Structural Elucidation

Hypofolin A (**1**), colorless needles, has a molecular formula of $C_{22}H_{30}O_4$ with eight indices of hydrogen deficiency, based on the sodiated molecular ion peak $[M + Na]^+$ at *m/z* 381.2034 (calc. 381.2036) in the HR-ESI-MS. Absorption bands at 1749, 1728, and 1644 cm^{-1} in the IR spectrum were suggestive of the presence of ester carbonyl, ketone, and olefinic groups, respectively. The ^{1}H-NMR spectroscopic data (*Table 1*) exhibited the presence of three methyl groups (δ(H) 0.57 (*s*, 3 H), 1.23 (*s*, 3 H), 1.71 (*s*, 3 H)),

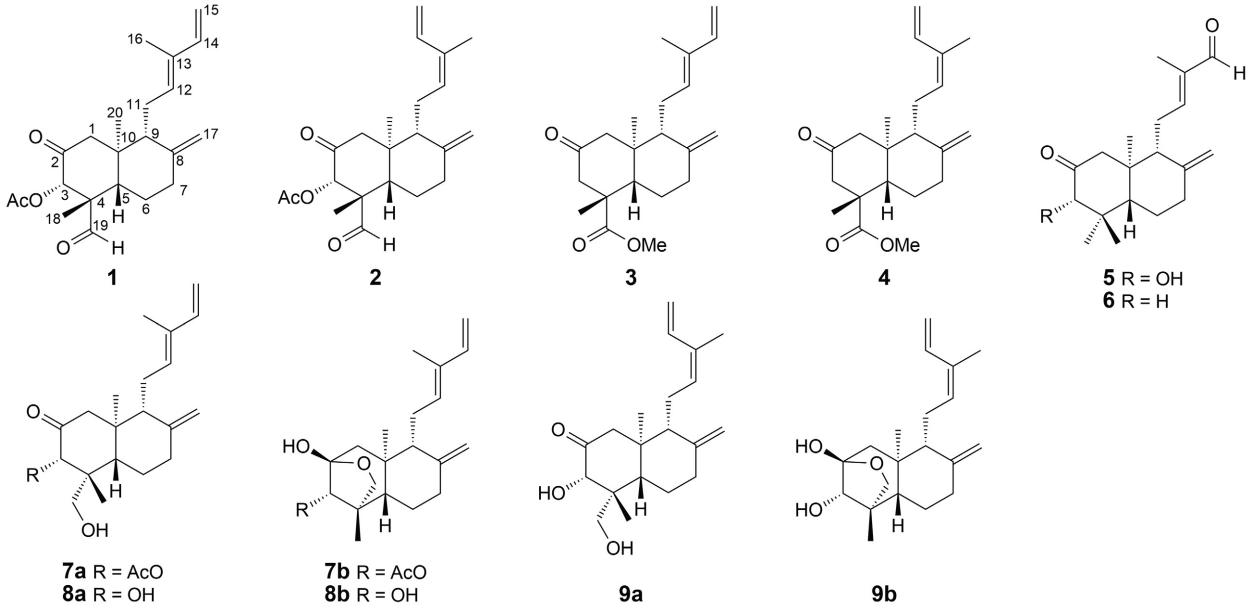


Figure 1. Structures of compounds **1** – **6**, **7a**/**7b**, **8a**/**8b**, and **9a**/**9b**.

an acetyl methyl (δ(H) 2.05 (*s*, 3 H)), an exocyclic methylene (δ(H) 4.85 (br. *s*, 1 H); 4.50 (br. *s*, 1 H)), an olefinic methine (δ(H) 5.35(*t*, J = 6.5 Hz, 1 H)), an oxygenated methine (δ(H) 5.28 (*s*, 1 H)), a formyl group (δ(H) 9.60 (*s*, 1 H)), a terminal vinyl group (δ(H) 6.26 (*dd*, J = 10.8, 17.4 Hz, 1 H), 5.01 (*d*, J = 17.4 Hz, 1 H), 4.81 (*d*, J = 10.8 Hz, 1 H)). The ^{13}C-NMR and DEPT spectra exhibited 22 carbon resonances (*Table 2*) attributable to three methyl, six methylene (two olefinic), and five methine groups (one oxygenated and two olefinic), four quaternary carbons (two olefinic), one ketone carbonyl, one aldehyde, and one acetoxy group. The above NMR spectroscopic data of **1** revealed that its structural features were similar to those of the *ent*-labdane diterpenoid 3α-acetoxy-*ent*-labda-8(17),12*E*,14-trien-19-ol.[18] The major difference between **1** and 3α-acetoxy-*ent*-labda-8(17),12*E*,14-trien-19-ol included that one ketone carbonyl (δ(C) 203.0) and one formyl group (δ(C) 202.4) in **1** replaced one methylene and the hydroxymethyl group in the latter, respectively. HMBCs (*Figure 2*) from H–C(3) (δ(H) 5.28 (*s*)) and CH_2(1) (δ(H) 2.78 (*d*, J = 12.5) and 2.57 (*d*, J = 12.5)) to C(2) (δ(C) 203.0) allowed the location of the ketone carbonyl at C(2). The assignment of the formyl group at C(4) was validated by the correlations of H–C(19) (δ(H) 9.60 (*s*)) with C(3) (δ(C) 81.6), C(4) (δ(C) 57.3), and C(5) (δ(C) 55.8) in the HMBC spectrum. With the help of a ROESY experiment, the relative configuration of **1** was deduced. The ROESY correlations of H–C(3)/Me(18), H–C(5)/Me(18), H–C(5)/H–C(9), and H–C(19)/Me(20) (*Figure 2*) indicated the β-orientations of H–C(5), H–C(9), and Me(18) as well as the α-orientations of AcO–C(3) and Me(20). The (*E*)-configuration of Δ^{12} was defined by the ROESY correlation of H–C(12)/H–C(14). The absolute configuration of **1** was determined by a single-crystal X-ray diffraction analysis using CuK_α radiation. The *Flack* parameter (−0.06(4)) permitted assignment of the absolute configuration as (3*S*,4*S*,5*S*,9*R*,10*S*) (*Figure 3*). Thus, structure of **1** was established as (3*S*,4*S*,12*E*)-3-acetoxy-2-oxo-*ent*-labda-8(17),12,14-trien-19-al.

Hypofolin B (**2**) was isolated as a colorless oil. Its molecular formula was established as $C_{22}H_{30}O_4$ based on the sodium-adduct HR-ESI-MS ion at *m/z* 381.2029 (calc. 381.2036), consistent with eight indices of hydrogen deficiency. The ^{1}H- and ^{13}C-NMR spectroscopic data (*Tables 1* and *2*) of **2** were superimposable on those of **1**, indicating their structures to be closely related, with the exception of the configuration of double bond between C(12) and C(13). The correlations of CH_2(11) with H–C(14) and of H–C(12) with Me(16) in the ROESY spectrum proved the (12*Z*) configuration. The relative configurations of C(3), C(4), C(5), C(9), and C(10) in **2** were identical to those in **1** by analysis of the ROESY experiment. The ECD spectrum of **1** and **2** (*Figures S8* and *S16*, *Supporting Information*) were almost the same, which allowed the determination of the absolute configuration of **2** as that of **1**. Therefore, the structure of **2** was identified as (3*S*,4*S*,12*Z*)-3-acetoxy-2-oxo-*ent*-labda-8(17),12,14-trien-19-al.

Hypofolins C (**3**) and D (**4**) both showed the same molecular formula, $C_{21}H_{30}O_3$, as established by HR-ESI-MS at *m/z* 353.2082 ([M + Na]$^+$) and 353.2079

Table 1. ^{1}H-NMR spectroscopic data of **1** – **6** in (D_6)acetone

Position	**1**[a]	**2**[b]	**3**[a]	**4**[c]	**5**[c]	**6**[c]
1	2.78 (*d*, *J* = 12.5)	2.81 (*d*, *J* = 12.5)	2.43 (*d*, *J* = 13.9)	2.45 (*d*, *J* = 13.7)	2.64 (*d*, *J* = 12.2)	2.46 (*d*, *J* = 12.1)
	2.57 (*d*, *J* = 12.5)	2.64 (*d*, *J* = 12.5)	2.34 (*d*, *J* = 13.9)	2.34 (*d*, *J* = 13.7)	2.51 (*d*, *J* = 12.2)	2.32 (*d*, *J* = 12.1)
3	5.28 (*s*)	5.34 (*s*)	2.72 (*d*, *J* = 14.1)	2.73 (*d*, *J* = 14.1)	4.05 (*d*, *J* = 4.3)	2.47 (*d*, *J* = 12.0)
			2.27 (*d*, *J* = 14.1)	2.28 (*d*, *J* = 14.1)		2.33 (*d*, *J* = 12.0)
5	2.39 (*dd*, *J* = 12.6, 3.1)	2.46 (*dd*, *J* = 13.1, 2.9)	2.09 (*dd*, *J* = 12.5, 2.7)	2.03 – 2.12 (*m*)	2.02 (*dd*, *J* = 12.7, 2.6)	1.91 (*dd*, *J* = 12.9, 2.7)
6	1.90 – 1.96 (*m*)	1.97 – 2.03 (*m*)	2.07 – 2.15 (*m*)	2.07 – 2.15 (*m*)	1.87 – 1.94 (*m*)	1.87 – 1.94 (*m*)
	1.20 – 1.28 (*m*)	1.27 – 1.33 (*m*)	1.71 – 1.79 (*m*)	1.71 – 1.80 (*m*)	1.45 – 1.54 (*m*)	1.40 – 1.49 (*m*)
7	2.36 – 2.41 (*m*)	2.42 – 2.47 (*m*)	2.41 – 2.48 (*m*)	2.41 – 2.48 (*m*)	2.44 – 2.53 (*m*)	2.43 – 2.51 (*m*)
	1.98 – 2.07 (*m*)	2.07 – 2.13 (*m*)	2.03 – 2.11 (*m*)	2.02 – 2.10 (*m*)	2.17 (*td*, *J* = 13.1, 4.4)	2.12 – 2.19 (*m*)
9	2.15 – 2.23 (*m*)	2.22 (br. *d*, *J* = 10.6)	2.02 – 2.09 (*m*)	1.99 – 2.06 (*m*)	2.39 (br. *d*, *J* = 10.3)	2.03 (br. *d*, *J* = 10.2)
11	2.24 – 2.32 (*m*)	2.37 – 2.43 (*m*)	2.26 – 2.34 (*m*)	2.32 – 2.39 (*m*)	2.53 – 2.59 (*m*)	2.50 – 2.55 (*m*)
	2.16 – 2.23 (*m*)	2.29 – 2.34 (*m*)	2.21 – 2.25 (*m*)	2.25 – 2.31 (*m*)	2.46 – 2.52 (*m*)	2.44 – 2.49 (*m*)
12	5.35 (*t*, *J* = 6.5)	5.31 (*t*, *J* = 6.6)	5.40 (*t*, *J* = 6.5)	5.30 (*t*, *J* = 6.3)	6.51 (*t*, *J* = 6.0)	6.50 (*t*, *J* = 6.0)
14	6.26 (*dd*, *J* = 17.4, 10.8)	6.88 (*dd*, *J* = 17.3, 10.8)	6.31 (*dd*, *J* = 17.4, 10.7)	6.85 (*dd*, *J* = 17.3, 10.9)	9.35 (*s*)	9.34 (*s*)
15	5.01 (*d*, *J* = 17.4)	5.20 (*d*, *J* = 17.3)	5.05 (*d*, *J* = 17.4)	5.19 (*d*, *J* = 17.3)		
	4.81 (*d*, *J* = 10.8)	5.10 (*d*, *J* = 10.8)	4.86 (*d*, *J* = 10.7)	5.09 (*d*, *J* = 10.9)		
16	1.71 (*s*)	1.76 (*s*)	1.75 (*s*)	1.75 (*s*)	1.74 (*s*)	1.73 (*s*)
17	4.85 (br. *s*)	4.92 (br. *s*)	4.90 (br. *s*)	4.90 (br. *s*)	4.92 (br. *s*)	4.90 (br. *s*)
	4.50 (br. *s*)	4.58 (br. *s*)	4.53 (br. *s*)	4.55 (br. *s*)	4.53 (br. *s*)	4.51 (br. *s*)
18	1.23 (*s*)	1.30 (*s*)	1.34 (*s*)	1.35 (*s*)	1.17 (*s*)	1.07 (*s*)
19	9.60 (*s*)	9.66 (*s*)			0.67 (*s*)	0.83 (*s*)
20	0.57 (*s*)	0.63 (*s*)	0.57 (*s*)	0.57 (*s*)	0.74 (*s*)	0.75 (*s*)
3-OH					3.71 (*d*, *J* = 4.3)	
3-AcO	2.05 (*s*)	2.11 (*s*)				
19-MeO			3.57 (*s*)	3.57 (*s*)		

δ in ppm, *J* in Hz. [a] Recorded at 500 MHz; [b] Recorded at 800 MHz; [c] Recorded at 600 MHz.

CHEMISTRY & BIODIVERSITY

Table 2. ^{13}C-NMR spectroscopic data of **1** – **6** in (D_6)acetone (δ in ppm)

Position	**1**[a]	**2**[b]	**3**[a]	**4**[c]	**5**[c]	**6**[c]
1	52.2	52.1	54.4	54.3	51.6	56.6
2	203.0	202.9	206.8	206.8	210.9	209.9
3	81.6	81.6	51.5	51.5	82.7	54.3
4	57.3	57.2	48.3	48.3	45.6	39.4
5	55.8	55.8	54.8	54.8	53.9	54.9
6	25.0	24.9	26.2	26.2	24.4	24.9
7	37.8	37.8	38.4	38.4	37.9	38.0
8	147.4	147.3	148.1	148.0	148.1	148.3
9	55.6	55.7	56.7	56.9	56.4	56.5
10	45.2	45.2	44.0	44.1	45.7	45.2
11	24.6	23.5	23.9	23.0	25.2	25.2
12	133.7	131.3	133.9	131.7	155.5	155.7
13	134.9	132.9	134.5	132.6	140.0	139.8
14	142.5	134.7	142.4	134.7	194.9	194.9
15	110.8	114.1	110.5	113.9		
16	12.1	19.9	11.9	19.9	9.3	9.3
17	109.8	109.9	108.8	108.9	109.1	109.0
18	21.2	21.1	28.2	28.2	29.3	33.5
19	202.4	202.3	176.6	176.6	16.8	23.3
20	16.6	16.5	13.9	13.9	15.5	15.5
3-AcO	170.1	170.0				
	20.4	20.3				
19-MeO			51.9	51.8		

[a] Recorded at 125 MHz; [b] Recorded at 200 MHz; [c] Recorded at 150 MHz.

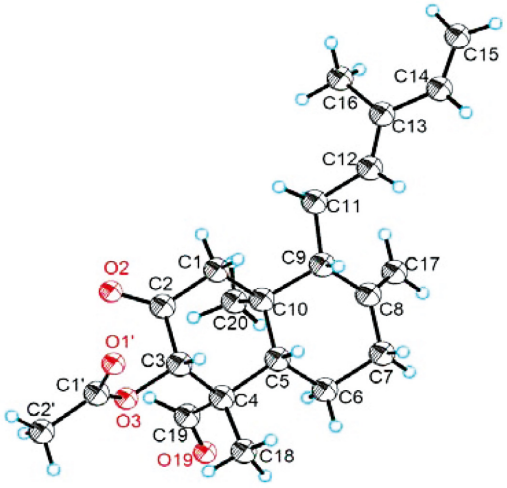


Figure 3. X-Ray crystallography structure of **1**.

([*M* + Na]$^+$), respectively, corresponding to seven indices of hydrogen deficiency. For compound **3**, the ^{1}H- and ^{13}C-NMR spectroscopic data (*Tables 1* and *2*) of **3** were closely comparable to those of **1**. Important differences involved the absence of each an acetyl and an oxygenated methine group and the presence of one methylene moiety at C(3), and that one methyl ester carbonyl group (δ(H) 3.57 (*s*); δ(C) 51.9 and 176.6) in **3** in place of the formyl residue of C(19). This deduction was corroborated by HMBCs of CH_2(3) (δ(H) 2.72 (*d*, *J* = 14.1) and 2.27 (*d*, *J* = 14.1)) to C(2) (δ(C) 206.8), C(4) (δ(C) 48.3), and C(5) (δ(C) 54.8), and of CH_2(3), H–C(5) (δ(H) 2.09 (*dd*, *J* = 12.5, 2.7)), Me(18) (δ(H) 1.34 (*s*)), and MeO (δ(H) 3.57 (*s*)) to C(19) (δ(C) 176.6). The ROESY correlations of Me(18)/H–C(5) and Me(18)/H–C(9) suggested that these protons were β-oriented. Meanwhile, Me(20) had an α-orientation based on the ROESY correlation of Me(20)/CH_2(11). The (*E*)-configuration of Δ^{12} was confirmed by a ROESY correlation between H–C(14) and H–C(12). Therefore, compound **3** was identified as methyl (4*R*,12*E*)-2-oxo-*ent*-labda-8(17),12,14-trien-19-oate. For compound **4**, the planar structure, methyl 2-oxo-*ent*-labda-8(17),12,14-trien-19-oate, was determined by comparison of its 1D-NMR spectroscopic data with those of **3**, and confirmed by further analysis of the HSQC, HMBC, and ^{1}H,^{1}H-COSY spectra. ROSEY spectrum showed that the difference between these two compounds was the configuration of Δ^{12}. The (*Z*)-configuration of Δ^{12} in **4** was recognized by the ROSEY correlation of H–C(12)/CH_2(16). Thus, the structure of **4** was identified as methyl (4*R*,12*Z*)-2-oxo-*ent*-labda-8(17),12,14-trien-19-oate.

The molecular formula $C_{19}H_{28}O_3$, with six indices of hydrogen deficiency, was assigned to hypofolin E (**5**) by the ^{13}C-NMR data and the HR-ESI-MS ion at

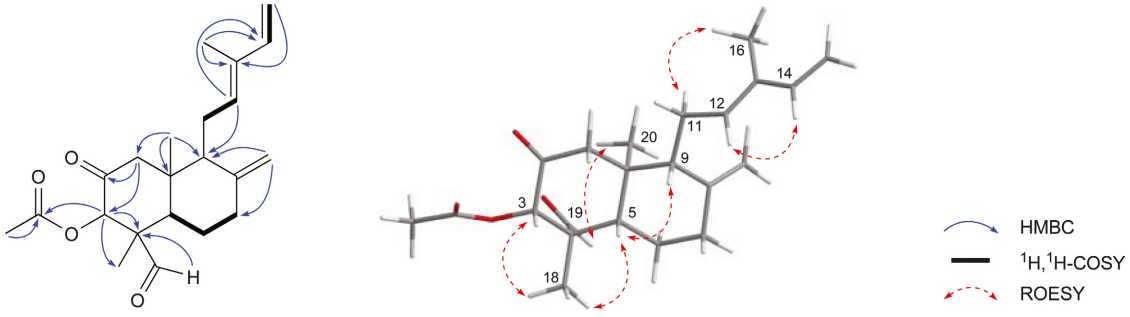


Figure 2. Key 2D-NMR correlations of **1**.

m/z 327.1927 ([*M* + Na]$^+$; calc. 327.1931). The presence of hydroxy (3432 cm^{-1}) and carbonyl (1714 cm^{-1}) functionalities were evident from their characteristic IR absorptions. In the ^{1}H-NMR spectrum (*Table 1*), four methyl protons (δ(H) 0.67 (*s*, 3 H), 0.74 (*s*, 3 H), 1.17 (*s*, 3 H), and 1.74 (*s*, 3 H)), one oxygenated methine proton (δ(H) 4.05 (*d*, *J* = 4.3 Hz, 1 H)), one trisubstituted olefinic proton (δ(H) 6.51 (*t*, *J* = 6.0 Hz, 1 H)), an exocyclic methylene group (δ(H) 4.92 (br. *s*, 1 H) and 4.53 (br. *s*, 1 H)), and one formyl proton (δ(H) 9.35 (*s*, 1 H)) were observed. The ^{13}C-NMR spectrum (*Table 2*) of **5** showed 19 signals, including those for four methyl, five methylene (one exomethylene at δ(C) 109.1), and five methine groups (one olefinic at δ(C) 155.5, one oxygenated C-atom at δ(C) 82.7, and one formyl group at δ(C) 194.9), and five nonprotonated carbons (one ketone carbonyl at δ(C) 210.9 and two olefinic at δ(C) 148.1 and 140.0). The aforementioned data implied that compound **5** was an *ent*-norlabdane-type diterpenoid. The ^{1}H- and ^{13}C-NMR spectroscopic data of **5** closely resembled those of **1**; however, **5** contained the signals of a formyl group (δ(H) 9.35 (*s*); δ(C) 194.9) and lacked the resonances for a terminal vinyl motif. The HMBCs (*Figure 4*) of H–C(14) (δ(H) 9.35 (*s*)) with C(12) (δ(C) 155.5), C(13) (δ(C) 140.0), and C(16) (δ(C) 9.3) indicated that the terminal moiety attached to C(13) in **1** was replaced by a formyl unit in **5**. The ROESY correlations of H–C(3)/H–C(5), H–C(3)/Me(18), H–C(5)/H–C(9), and Me(20)/CH$_2$(11) indicated the α-orientations of 3-OH and Me(20) and the β-orientations of Me(18), H–C(5), and H–C(9). Additionally, the ROESY correlation (*Figure 4*) of H–C(12)/H–C(14) revealed that Δ^{12} had (*E*)-configuration. Compound **5** had (3*S*,4*S*,5*S*,9*R*,10*S*) configurations due to the similar ECD spectra between **1** and **5** (*Figures S8* and *S38*, *Supporting Information*). Thus, the structure of compound **5** was defined as (3*S*,12*E*)-3-hydroxy-2-oxo-15-nor-*ent*-labda-8(17),12-dien-14-al.

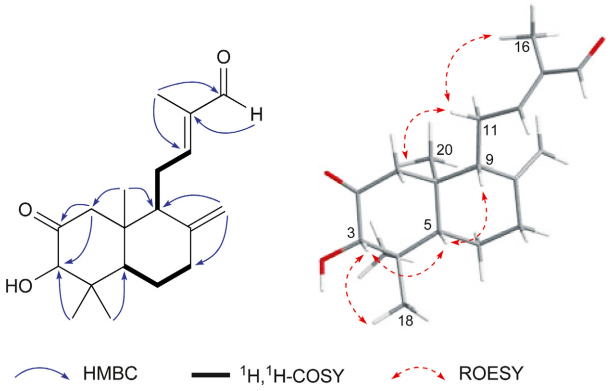


Figure 4. Key 2D-NMR correlations of **5**.

The molecular formula of hypofolin F (**6**) was established as $C_{19}H_{28}O_2$ based on the HR-ESI-MS data (*m/z* 311.1976 ([*M* + Na]$^+$; calc. 311.1982)), indicating six indices of hydrogen deficiency. The existence of carbonyl (1709 cm^{-1}) and olefinic (1642 cm^{-1}) functionalities was specified from IR spectrum. Analysis of ^{1}H- and ^{13}C-NMR spectroscopic data of **6** (*Tables 1* and *2*) revealed that its structure was also an *ent*-norlabdane-type diterpenoid. Compared with **5**, the carbon resonance of C(3) was shifted upfield from δ(C) 82.7 to δ(C) 54.3 in **6**, indicating the presence of a C(3) methylene in **6** rather than an oxygenated methine in **5**. This inference was evidenced by the key HMBCs of CH$_2$(3) (δ(H) 2.47 (*d*, *J* = 12.0) and 2.33 (*d*, *J* = 12.0)) with C(2) (δ(C) 209.9), C(4) (δ(C) 39.4), C(18) (δ(C) 33.5), and C(19) (δ(C) 23.3). The ROESY experiment confirmed that **6** displayed the same relative configuration as that of **5**. Accordingly, compound **6** was assigned as (12*E*)-2-oxo-15-nor-*ent*-labda-8(17),12-dien-14-al.

Hypofolins G (**7a**) and H (**7b**) were obtained as an interconverting mixture in a ratio of 3:2. The same molecular formula of $C_{22}H_{32}O_4$ (seven indices of hydrogen deficiency) was assigned to **7a** and **7b** by a pseudomolecular ion at *m/z* 383.2194 ([*M* + Na]$^+$; calc. 383.2198) in positive HR-ESI-MS spectrum. IR absorption bands at 1730 and 3442 cm^{-1} suggested the presence of carbonyl and hydroxy groups, respectively. Concretely, for the major isomer **7a**, the ^{1}H-NMR spectrum (*Table 3*) displayed the signals for an oxymethylene unit at δ(H) 3.56 (*dd*, *J* = 11.8, 5.3 Hz, 1 H), 3.50 (*dd*, *J* = 11.8, 5.3 Hz, 1 H), an oxymethine group at δ(H) 5.08 (*s*, 1 H), an olefinic proton at δ(H) 5.44 (*t*, *J* = 6.5 Hz, 1 H), a terminal vinyl group at δ(H) 6.32 (*dd*, *J* = 17.3, 10.9 Hz, 1 H), 5.05 (*d*, *J* = 17.3 Hz, 1 H), 4.86 (*d*, *J* = 10.9 Hz, 1 H), an exocyclic methylene moiety at δ(H) 4.89 (br. *s*, 1 H) and 4.56 (br. *s*, 1 H), three tertiary methyl groups at δ(H) 1.77 (*s*, 3 H), 1.20 (*s*, 3 H), and 0.84 (*s*, 3 H), and an acetyl methyl at δ(H) 2.11(*s*, 3 H). The ^{13}C-NMR and DEPT spectra (*Table 3*) exhibited 22 carbon resonances comprising three methyl, seven methylene (including two olefinic and one oxygenated), and five methine groups (including two olefinic and one oxygenated), five quaternary carbons (including two olefinic and one ketone carbonyl), and one acetoxy group. On the basis of spectroscopic data analysis, compound **7a** was deduced to be similar to compound **1**. Comparing the ^{1}H- and ^{13}C-NMR spectroscopic data of **7a** with those of **1** showed that the formyl group was not present, instead, a hydroxymethyl unit at C(4) was observed in **7a**, which was further confirmed by HMBCs from CH$_2$(19) (δ(H) 3.56 (*dd*, *J* = 11.8, 5.3), 3.50 (*dd*, *J* = 11.8, 5.3)) to C(4) (δ(C)

Table 3. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectroscopic data of **7a**/**7b**, **7c**, and **7d** in (D_6)acetone (δ in ppm, J in Hz)

Position	**7a**		**7b**		**7c**		**7d**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	2.61 (*d*, *J* = 12.6)	52.8	1.96 (*d*, *J* = 12.8)	48.7	2.67 (*d*, *J* = 12.6)	52.6	2.20 (*d*, *J* = 13.3)	45.5
	2.49 (*d*, *J* = 12.6)		1.86 (*d*, *J* = 12.8)		2.52 (*d*, *J* = 12.6)		1.67 (*d*, *J* = 13.3)	
2		203.6		105.8		203.2		108.2
3	5.08 (*s*)	84.2	4.72 (*s*)	83.1	5.12 (*s*)	83.2	4.77 (*s*)	83.2
4		47.9		47.9		46.5		47.8
5	2.15 – 2.21 (*m*)	55.3	1.72 – 1.79 (*m*)	54.6	2.25 – 2.32 (*m*)	54.7	1.73 – 1.80 (*m*)	54.8
6	1.92 – 1.96 (*m*)	25.6	1.68 – 1.73 (*m*)	23.9	1.96 – 2.02 (*m*)	25.4	1.67 – 1.73 (*m*)	23.9
	1.74 – 1.81 (*m*)		1.48 – 1.56 (*m*)		1.62 – 1.71 (*m*)		1.49 – 1.54 (*m*)	
7	2.42 – 2.48 (*m*)	38.6	2.42 – 2.48 (*m*)	37.8	2.43 – 2.50 (*m*)	38.4	2.41 – 2.47 (*m*)	37.8
	2.04 – 2.10 (*m*)		2.10 – 2.17 (*m*)		2.06 – 2.15 (*m*)		2.10 – 2.17 (*m*)	
8		148.3		148.6		147.9		148.7
9	2.14 – 2.23 (*m*)	57.3	2.00 – 2.07 (*m*)	58.0	2.18 – 2.25 (*m*)	57.2	2.01 – 2.08 (*m*)	58.0
10		45.0		40.0		45.0		39.9
11	2.26 – 2.34 (*m*)	24.0	2.26 – 2.34 (*m*)	24.9	2.27 – 2.35 (*m*)	24.2	2.29 – 2.37 (*m*)	25.0
	2.26 – 2.34 (*m*)		2.26 – 2.34 (*m*)		2.27 – 2.35 (*m*)		2.29 – 2.37 (*m*)	
12	5.44 (*t*, *J* = 6.5)	134.0	5.44 (*t*, *J* = 6.5)	134.4	5.43 (*t*, *J* = 6.4)	133.8	5.43 (*t*, *J* = 6.5)	134.5
13		134.5		134.3		134.6		134.2
14	6.32 (*dd*, *J* = 17.3, 10.9)	142.5	6.32 (*dd*, *J* = 17.3, 10.9)	142.5	6.31 (*dd*, *J* = 17.3, 10.9)	142.4	6.31 (*dd*, *J* = 17.4, 10.8)	142.5
15	5.05 (*d*, *J* = 17.3)	110.5	5.05 (*d*, *J* = 17.3)	110.4	5.06 (*d*, *J* = 17.3)	110.6	5.05 (*d*, *J* = 17.4)	110.4
	4.86 (*d*, *J* = 10.9)		4.86 (*d*, *J* = 10.9)		4.86 (*d*, *J* = 10.9)		4.85 (*d*, *J* = 10.8)	
16	1.77 (*s*)	12.0	1.77 (*s*)	12.0	1.77 (*s*)	12.0	1.76 (*s*)	12.0
17	4.89 (br. *s*)	109.1	4.89 (br. *s*)	109.5	4.92 (br. *s*)	109.4	4.86 (br. *s*)	109.7
	4.56 (br. *s*)		4.56 (br. *s*)		4.58 (br. *s*)		4.55 (br. *s*)	
18	1.20 (*s*)	23.5	0.84 (*s*)	15.4	1.23 (*s*)	23.7	0.86 (*s*)	15.3
19	3.56 (*dd*, *J* = 11.8, 5.3)	63.5	3.75 (*d*, *J* = 8.0)	70.7	4.02 (*d*, *J* = 12.0)	64.5	3.84 (*d*, *J* = 8.0)	71.8
	3.50 (*dd*, *J* = 11.8, 5.3)		3.51 (*d*, *J* = 8.0)		3.96 (*d*, *J* = 12.0)		3.60 (*d*, *J* = 8.0)	
20	0.84 (*s*)	15.8	1.01 (*s*)	16.5	0.80 (*s*)	15.2	1.01 (*s*)	16.6
MeO							3.33 (*s*)	51.1
19-OH	3.27 (*t*, *J* = 5.3)							
3-AcO		170.3		170.8		170.2		170.3
	2.11 (*s*)	20.5	2.13 (*s*)	20.9	2.09 (*s*)	20.4	2.09 (*s*)	20.9
19-AcO						170.5		
					1.98 (*s*)	20.7		

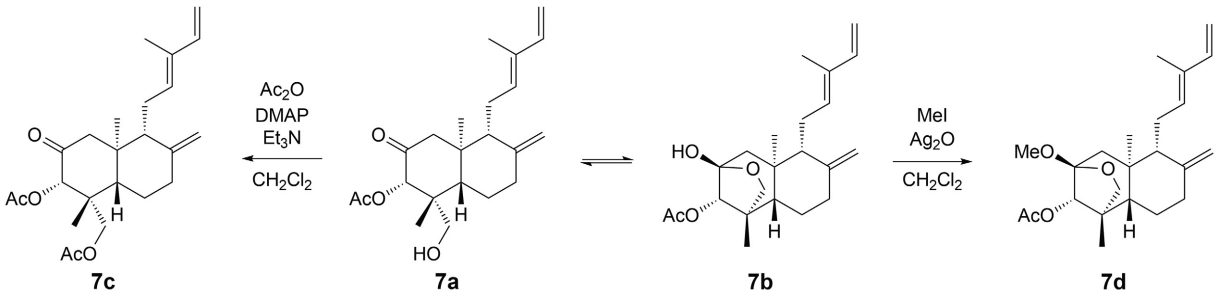
CHEMISTRY & BIODIVERSITY

47.9), C(5) (δ(C) 55.3), and C(18) (δ(C) 23.5). The relative configuration of **7a** was determined to be consistent with that of **1** by analysis of the ROESY experiment. Therefore, compound **7a** was established as (3*S*,4*R*,12*E*)-3-acetoxy-19-hydroxy-*ent*-labda-8(17),12,14-trien-2-one.

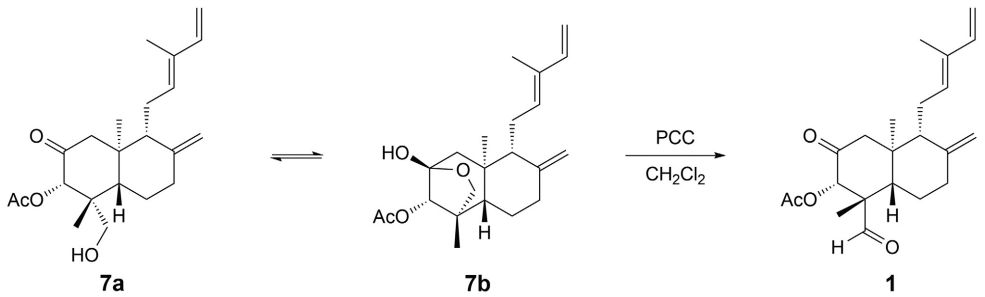
The ^{1}H- and ^{13}C-NMR spectroscopic data (*Table 3*) of **7b** were similar to those of **7a**, with the exception that the resonance of a hemiketal carbon at C(2) (δ(C) 105.8) in **7b** replaced the carbon signal for a ketone carbonyl in **7a**, and that the chemical shift of C(19) in **7a** was deshielded by 7.2 ppm when compared with that in **7b**, suggesting that a five-membered hemiketal ring was formed between C(2) and C(19). This proposed structure was recognized by HMBCs from CH_2(19) (δ(H) 3.75 (*d*, J = 8.0) and 3.51 (*d*, J = 8.0)), CH_2(1) (δ(H) 1.96 (*d*, J = 12.8) and 1.86 (*d*, J = 12.8)), and H–C(3) (δ(H) 4.72 (*s*)) to C(2) (δ(C) 105.8). The α-orientation of 3-OH was verified by the ROESY correlations of CH_2(19) with Me(20). Consequently, the structure of compound **7b** was defined as (2*S*,3*S*,4*R*,12*E*)-3-acetoxy-2,19-epoxy-*ent*-labda-8(17),12,14-trien-2-ol.

Interestingly, only the acetyl product **7c** was obtained by aceylation of **7a** and **7b** with Ac_2O. Furthermore, methylation of **7a** and **7b** with CH_3I afforded only the ketal product **7d**. These two chemical transformations (*Scheme 1*) confirmed that **7a** and **7b** were an interconverting mixture of two isomers between ketone and hemiketal types. Subsequently, to determine the absolute configurations of compounds **7a** and **7b**, compound **1** was synthesized from **7a**/**7b** *via* oxidation reaction with PCC (*Scheme 2*). The 1D-NMR and ECD spectra, and specific rotation value of synthetic **1** coincided with those of natural **1**. Therefore, the absolute configurations of **7a** and **7b** were determined to be (3*S*,4*R*,5*S*,9*R*,10*S*) and (2*S*,3*S*,4*R*,5*S*,9*R*,10*S*), respectively.

Hypofolins I (**8a**) and J (**8b**) were also isolated as a pair of interchangeable compounds with a ratio of 5:2, which possessed the same molecular formula of $C_{20}H_{30}O_3$, as established by the HR-ESI-MS data (*m/z* 341.2086 ([M + Na]$^+$; calc. 341.2087)). The IR spectrum of **8a**/**8b** indicated the presence of hydroxy (3426 cm^{-1}) and carbonyl (1715 cm^{-1}) groups. The 1D-NMR spectra (*Table 4*) of **8a**/**8b** were similar to those of **7a**/**7b**, except for the presence of a pair of hydroxy group instead of a pair of acetoxy group, which was confirmed by HMBCs. Concretely, in **8a**, HMBCs of H–C(3) (δ(H) 4.43 (*s*)) with C(2) (δ(C) 210.8), C(4) (δ(C) 49.6), C(18) (δ(C) 24.7), and C(19) (δ(C) 64.3) confirmed a hydroxy group at C(3) in **8a**. In a similar method, the 3-OH in compoud **8b** was confirmed on the basis of HMBCs from H–C(3) (δ(H) 3.70) to C(2) (δ(C) 107.2), C(5) (δ(C) 55.0), and C(19) (δ(C) 71.0). The similar ROESY correlations of **8a**/**8b** and **7a**/**7b** clarified two pairs of compounds sharing the same configurations. Thus, the structures of **8a**/**8b** were deduced as (3*S*,4*R*,12*E*)-3,19-dihydroxy-*ent*-labda-



Scheme 1. Compounds **7a**/**7b** were acetylated and methylated to afford compounds **7c**/**7d**.



Scheme 2. Compounds **7a**/**7b** were oxidized to afford compound **1**.

Table 4. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectroscopic data of **8a**/**8b** and **9a**/**9b** in (D_6)acetone (δ in ppm, J in Hz)

Position	**8a**		**8b**		**9a**		**9b**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	2.77 (*d*, *J* = 12.6) 2.49 (*d*, *J* = 12.6)	52.7	2.43 (*d*, *J* = 12.6) 2.04 (*d*, *J* = 12.6)	48.9	2.79 (*d*, *J* = 12.6) 2.49 (*d*, *J* = 12.6)	52.7	2.45 (*d*, *J* = 12.6) 2.05 (*d*, *J* = 12.6)	48.9
2		210.8		107.2		210.8		107.2
3	4.43 (*s*)	84.1	3.70 (*s*)	82.3	4.42 (*s*)	84.1	3.69 (*s*)	82.3
4		49.6		48.5		49.6		48.4
5	1.92 – 2.00 (*m*)	55.0	1.56 – 1.63 (*m*)	55.0	1.91 – 1.98 (*m*)	55.1	1.55 – 1.61 (*m*)	55.0
6	1.91 – 1.96 (*m*) 1.68 – 1.76 (*m*)	25.3	1.57 – 1.65 (*m*) 1.48 – 1.56 (*m*)	24.1	1.90 – 1.96 (*m*) 1.68 – 1.76 (*m*)	25.3	1.57 – 1.65 (*m*) 1.46 – 1.54 (*m*)	24.1
7	2.38 – 2.47 (*m*) 2.01 – 2.09 (*m*)	38.6	2.38 – 2.47 (*m*) 2.01 – 2.09 (*m*)	38.1	2.38 – 2.46 (*m*) 2.00 – 2.10 (*m*)	38.6	2.38 – 2.46 (*m*) 2.00 – 2.10 (*m*)	38.1
8		148.1		148.8		148.0		148.8
9	2.03 – 2.11 (*m*)	57.4	1.89 – 1.97 (*m*)	58.3	2.02 – 2.10 (*m*)	57.7	1.89 – 1.96 (*m*)	58.5
10		45.2		40.3		45.3		40.4
11	2.25 – 2.33 (*m*) 2.25 – 2.33 (*m*)	24.4	2.32 – 2.40 (*m*) 2.32 – 2.40 (*m*)	25.1	2.30 – 2.38 (*m*) 2.30 – 2.38 (*m*)	23.4	2.41 – 2.48 (*m*) 2.41 – 2.48 (*m*)	24.2
12	5.57 (*t*, *J* = 6.5)	134.2	5.57 (*t*, *J* = 6.5)	134.8	5.44 (*t*, *J* = 6.5)	131.9	5.44 (*t*, *J* = 6.5)	132.6
13		134.6		134.3		132.7		132.3
14	6.47 (*dd*, *J* = 17.4, 10.8)	142.4	6.47 (*dd*, *J* = 17.4, 10.8)	142.5	6.96 (*dd*, *J* = 17.2, 10.8)	134.7	6.96 (*dd*, *J* = 17.2, 10.8)	134.7
15	5.18 (*d*, *J* = 17.4) 4.99 (*d*, *J* = 10.8)	111.1	5.15 (*d*, *J* = 17.4) 4.97 (*d*, *J* = 10.8)	110.8	5.29 (*d*, *J* = 17.2) 5.21 (*d*, *J* = 10.8)	114.4	5.21 (*d*, *J* = 17.2) 5.13 (*d*, *J* = 10.8)	114.2
16	1.80 (*s*)	12.5	1.76 (*s*)	12.4	1.82 (*s*)	20.4	1.80 (*s*)	20.4
17	4.96 (br. *s*) 4.63 (br. *s*)	109.5	4.97 (br. *s*) 4.67 (br. *s*)	109.8	4.97 (br. *s*) 4.67 (br. *s*)	109.6	4.98 (br. *s*) 4.71 (br. *s*)	110.0
18	1.58 (*s*)	24.7	1.24 (*s*)	17.1	1.58 (*s*)	24.7	1.25 (*s*)	17.1
19a	4.06 (*d*, *J* = 11.4) 3.77 (*d*, *J* = 11.4)	64.3	4.03 (*d*, *J* = 7.6) 3.87 (*d*, *J* = 7.6)	71.0	4.06 (*d*, *J* = 11.4) 3.77 (*d*, *J* = 11.4)	64.3	4.03 (*d*, *J* = 7.6) 3.86 (*d*, *J* = 7.6)	70.9
20	1.00 (*s*)	16.0	1.15 (*s*)	16.0	1.00 (*s*)	16.0	1.14 (*s*)	16.0

8(17),12,14-trien-2-one and (2*S*,3*S*,4*R*,12*E*)-2,19-epoxy-*ent*-labda-8(17),12,14-trien-2,3-diol, respectively.

Hypofolins K (**9a**) and L (**9b**) were obtained as an interchangeable mixture in a ratio of 5:2. The molecular formula of **9a**/**9b** was determined to be $C_{20}H_{30}O_3$ by the HR-ESI-MS data (*m/z* 341.2089 ([*M* + Na]$^+$; calc. 341.2087)). The ^{1}H- and ^{13}C-NMR spectroscopic data (*Table 4*) of **9a**/**9b** shared significant similarities to those of **8a**/**8b**, aside from the Δ^{12} configurations of the C(10) side chain moiety. The (*Z*)-configurations of Δ^{12} in **9a**/**9b** were confirmed by ROESY correlations of H–C(14)/CH_2(11). Accordingly, compounds **9a** and **9b** were established as (3*S*,4*R*,12*Z*)-3,19-dihydroxy-*ent*-labda-8(17),12,14-trien-2-one and (2*S*,3*S*,4*R*,12*Z*)-2,19-epoxy-*ent*-labda-8(17),12,14-trien-2,3-diol, respectively.

Biological Studies

Compounds **1**, **3**, **5**, **7a**/**7b**, **7c**, **7d**, **8a**/**8b**, and **9a**/**9b** were assayed *in vitro* for their cytotoxic activity against HL-60, SMMC-7721, A-549, MCF-7, and SW-480 cell lines by the MTS method. As a result, compound **1** displayed weak cytotoxicity against the SMMC-7721 cell line with an IC_{50} value of 31.40 μM. The other compounds had no remarkable effect with IC_{50} values of more than 40 μM. Compounds **1**, **3**, **5**, **7a**/**7b**, **7c**, **7d**, **8a**/**8b**, and **9a**/**9b** were further evaluated for their capability to inhibit NO production in LPS-stimulated RAW 264.7 cell with L-NMMA as positive control. Unfortunately, all compounds exhibited inhibition percentages of less than 50% at the concentration of 25 μM.

Conclusions

Twelve new *ent*-labdane diterpenoids, hypofolins A – F (**1** – **6**) and hypofolins G – L (**7a**/**7b**, **8a**/**8b**, and **9a**/**9b**), were isolated from the roots of *Hypoestes phyllostachya* 'Pink Splash'. The absolute configurations of **1**, **2**, **5**, and **7a**/**7b** were determinrd by single crystal X-ray diffraction and ECD analysis, as well as chemical transformations. Compounds **7a**/**7b**, **8a**/**8b**, and **9a**/**9b** were isolated as three interconverting pairs of two isomers between ketone and hemiketal types each. Apart from this, compound **1** showed weak cytotoxicity

against the SMMC-7721 cell line with IC_{50} value of 31.40 μM.

Experimental Section

General

Melting points were obtained on an *X-4 micro* melting point apparatus. Optical rotations were measured on a *Jasco P-1020* polarimeter. UV Spectra were obtained with the *Shimadzu UV2401PC* spectrometer, and IR spectra were measured on a *Tenor-27* spectrometer. ESI-MS and HR-ESI-MS were performed on a *UPLC-IT-TOF* or an *Agilent G6230* time-of-flight spectrometer. The NMR spectra were recorded on a *Bruker Avance III 500*, *AV-600*, or *AV-800*, and the chemical shifts were referenced to tetramethylsilane (TMS). Semipreparative HPLC was performed on an *Agilent 1260* liquid chromatography system equipped with a *Zorbax SB-C18* column (9.4 mm × 150 mm), MPLC was performed on a *Lisui EZ Purify III* System (*Shanghai Lisui Chemical Engineering Co., Ltd.*, Shanghai, P. R. China). Column chromatography (CC) was run on silica gel (200 – 300 mesh; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China) and *Sephadex LH-20* (*Pharmacia Fine Chemical Co., Ltd.*). TLC was performed on silica gel *GF254* (SiO_2; *Qingdao Haiyang Chemical Factory*, Qingdao, P. R. China), and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH. All solvents were distilled prior to use.

Plant Material

The root parts of *H. phyllostachya* 'Pink Splash' was collected from Kunming Botany Garden, Yunnan Province, P. R. China, in July 2016, and was identified by Prof. *Xiao Cheng* from Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (201606h01) was deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation

The air-dried and powdered root parts of *H. phyllostachya* 'Pink Splash' (5.0 kg) was extracted with 95% EtOH for three times at room temperature. The extract was filtered and concentrated under reduced pressure, then mixed with H_2O (300 ml) and subjected to solvent using AcOEt. The AcOEt portion (100 g) was fractionated by CC on MPLC (*MCI* gel), eluted with MeOH/H_2O (70:30 to 95:5), giving eleven fractions (*Fr. 1* – *Fr. 11*). *Fr. 2* (1.2 g) was purified by CC (silica gel, $CHCl_3$/acetone 100:1) to give three subfractions (*Fr. 2.1* – *Fr. 2.3*). Subfraction *Fr. 2.3* (25.0 mg) was separated by semipreparative HPLC (MeOH/H_2O 70:30) to give **5** (5.1 mg) and **6** (1.3 mg). *Fr. 3* (5.2 g) was separated using a silica gel CC and was eluted with a gradient solvent mixture of CH_3Cl/acetone (100:1, 50:1, 25:1, 9:1) to yield four subfractions (*Fr. 3.1* – *Fr. 3.4*), based on TLC analysis. Further purification of subfraction *Fr. 3.2* (970 mg) *via* silica gel CC eluted with petroleum ether/acetone (9:1) yielded a compound mixture (203 mg) which was further purified by silica gel CC (petroleum ether/AcOEt 9:1) to yield the mixture of **7a** and **7b** (83 mg). *Fr. 3.4* (92 mg) was further separated by semipreparative HPLC, eluting with MeCN/H_2O (45:55), to obtain mixtures **8a**/**8b** (5.4 mg) and **9a**/**9b** (4.2 mg). *Fr. 4* (7.8 g) was subjected to a silica gel CC (petroleum ether/acetone 50:1 to 4:1) to obtain nine subfractions (*Fr. 4.1* – *Fr. 4.9*). The purification of *Fr. 4.3* (34 mg) carried on a semi-preparative HPLC (MeOH/H_2O 73:27) to afford **3** (4.8 mg) and **4** (1.5 mg). Compound **1** (17.1 mg) was isolated from *Fr. 4.4* (28 mg) by silica gel CC (petroleum ether/AcOEt 9:1). *Fr. 4.5* (18 mg) was separated by semi-preparative HPLC with MeCN/H_2O (56:44) as mobile phase to obtain **2** (1.6 mg).

Hypofolin A (= **(3*S*,4*S*,12*E*)-3-Acetoxy-2-oxo-*ent*-labda-8(17),12,14-trien-19-al**; **1**). Colorless crystal. M.p. 179.6–181.3 °C. $[\alpha]_D^{23.7} = -39.3$ (c = 0.20, MeOH). UV (MeOH): 229 (4.36). ECD (MeOH): $\Delta\varepsilon$ 207 + 11.8, $\Delta\varepsilon$ 294 – 2.06. IR (KBr): 3433, 2955, 2851, 1749, 1728, 1644, 1440, 1375, 1231, 1087, 1042, 994, 895. ^{1}H-(500 MHz) and ^{13}C-NMR (125 MHz): see *Tables 1* and *2*. ESI-MS (pos.): 381 ($[M + Na]^+$). HR-ESI-MS (pos.): 381.2034 ($[M + Na]^+$, $C_{22}H_{30}NaO_4^+$; calc. 381.2036).

Hypofolin B (= **(3*S*,4*S*,12*Z*)-3-Acetoxy-2-oxo-*ent*-labda-8(17),12,14-trien-19-al**; **2**). Colorless oil. $[\alpha]_D^{23.6} = -73.8$ (c = 0.06, MeOH). UV (MeOH): 203 (3.92), 223 (3.72). ECD (MeOH): $\Delta\varepsilon$ 201 + 22.2, $\Delta\varepsilon$ 294 – 2.47. IR (KBr): 3433, 2940, 2745, 1727, 1643, 1437, 1380, 1232, 1087, 1043, 900. ^{1}H- (800 MHz) and ^{13}C-NMR (200 MHz): see *Tables 1* and *2*. ESI-MS (pos.): 381 ($[M + Na]^+$). HR-ESI-MS (pos.): 381.2029 ($[M + Na]^+$, $C_{22}H_{30}NaO_4^+$; calc. 381.2036).

Hypofolin C (= **Methyl (4*R*,12*E*)-2-Oxo-*ent*-labda-8(17),12,14-trien-19-oate**; **3**). Colorless oil. $[\alpha]_D^{23.5} = -26.9$ (c = 0.16, MeOH). UV (MeOH): 203 (3.89), 224 (3.64). IR (KBr): 3435, 2955, 1725, 1643, 1439, 1384, 1240, 1201, 1144, 894, 780, 575. ^{1}H-(500 MHz) and ^{13}C-NMR (125 MHz): see *Tables 1* and *2*. ESI-MS (pos.): 353 ($[M + Na]^+$). HR-ESI-MS (pos.): 353.2082 ($[M + Na]^+$, $C_{21}H_{30}NaO_3^+$; calc. 353.2087).

Hypofolin D (= **Methyl (4*R*,12*Z*)-2-Oxo-*ent*-labda-8(17),12,14-trien-19-oate**; **4**). Colorless oil. $[\alpha]_D^{23.8}$ = −49.0 (*c* = 0.10, MeOH). UV (MeOH): 203 (3.88), 224 (3.60). IR (KBr): 3434, 2954, 1724, 1642, 1446, 1384, 1240, 1201, 1144, 896, 780, 574. ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz): see *Tables 1* and *2*. ESI-MS (pos.): 353 ([*M* + Na]$^+$). HR-ESI-MS (pos.): 353.2079 ([*M* + Na]$^+$, $C_{21}H_{30}NaO_3^+$; calc. 353.2087).

Hypofolin E (= **(3*S*,12*E*)-3-Hydroxy-2-oxo-15-nor-*ent*-labda-8(17),12-dien-14-al**; **5**). Colorless oil. $[\alpha]_D^{23.8}$ = −6.1 (*c* = 0.23, MeOH). UV (MeOH): 203 (3.87), 228 (3.87). ECD (MeOH): Δε 206 + 12.3, Δε 290 − 1.82. IR (KBr): 3432, 2971, 2947, 2876, 1714, 1643, 1439, 1387, 1261, 1115, 1045, 892, 654. ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz): see *Tables 1* and *2*. ESI-MS (pos.): 327 ([*M* + Na]$^+$). HR-ESI-MS (pos.): 327.1927 ([*M* + Na]$^+$, $C_{19}H_{28}NaO_3^+$; calc. 327.1931).

Hypofolin F (= **(12*E*)-2-Oxo-15-nor-*ent*-labda-8(17),12-dien-14-al**; **6**). Colorless oil. $[\alpha]_D^{23.7}$ = −40.4 (*c* = 0.04, MeOH). UV (MeOH): 204 (4.03), 207 (3.95). IR (KBr): 3433, 2960, 2874, 1709, 1642, 1387, 1288, 1262, 894, 582, 556. ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz): see *Tables 1* and *2*. ESI-MS (pos.): 311 ([*M* + Na]$^+$). HR-ESI-MS (pos.): 311.1976 ([*M* + Na]$^+$, $C_{19}H_{28}NaO_2^+$; calc. 311.1982).

Hypofolin G (= **(3*S*,4*R*,12*E*)-3-Acetoxy-19-hydroxy-*ent*-labda-8(17),12,14-trien-2-one**; **7a**) and **H** (= **(2*S*,3*S*,4*R*,12*E*)-3-Acetoxy-2,19-epoxy-*ent*-labda-8(17),12,14-trien-2-ol**; **7b**). Colorless oil. $[\alpha]_D^{20.7}$ = −70.0 (*c* = 0.10, MeOH). UV (MeOH): 230 (4.65), 199 (4.33). IR (KBr): 3442, 3085, 2934, 2883, 2854, 1730, 1642, 1606, 1439, 1375, 1234, 1089, 1041, 952, 892, 840. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz): see *Table 3*. ESI-MS (pos.): 383 ([*M* + Na]$^+$). HR-ESI-MS (pos.): 383.2194 ([*M* + Na]$^+$, $C_{22}H_{32}NaO_4^+$; calc. 383.2198).

Hypofolin I (= **(3*S*,4*R*,12*E*)-3,19-Dihydroxy-*ent*-labda-8(17),12,14-trien-2-one**; **8a**) and **J** (= **(2*S*,3*S*,4*R*,12*E*)-2,19-Epoxy-*ent*-labda-8(17),12,14-trien-2,3-diol**; **8b**). Colorless oil. $[\alpha]_D^{20.7}$ = −18.6 (*c* = 0.10, MeOH). UV (MeOH): 203 (3.88), 229 (3.75). IR (KBr): 3426, 3086, 2942, 2882, 1715, 1642, 1446, 1386, 1256, 1203, 1133, 1096, 1043, 892, 650, 535. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz): see *Table 4*. ESI-MS (pos.): 341 ([*M* + Na]$^+$). HR-ESI-MS (pos.): 341.2086 ([*M* + Na]$^+$, $C_{20}H_{30}NaO_3^+$; calc. 341.2087).

Hypofolin K (= **(3*S*,4*R*,12*Z*)-3,19-Dihydroxy-*ent*-labda-8(17),12,14-trien-2-one**; **9a**) and **L** (= **(2*S*,3*S*,4*R*,12*Z*)-2,19-Epoxy-*ent*-labda-8(17),12,14-trien-2,3-diol**; **9b**). Colorless oil. $[\alpha]_D^{20.7}$ = −28.9 (*c* = 0.10, MeOH). UV (MeOH): 203 (3.99), 231 (3.86). IR (KBr): 3423, 3086, 2938, 2886, 1715, 1642, 1449, 1385, 1255, 1202, 1133, 1098, 1045, 895, 646, 534. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz): see *Table 4*. ESI-MS (pos.): 341 ([*M* + Na]$^+$). HR-ESI-MS (pos.): 341.2089 ([*M* + Na]$^+$, $C_{20}H_{30}NaO_3^+$; calc. 341.2087).

Acetylation of the **7a**/**7b** (**7c**)

To a solution of **7a**/**7b** (10 mg) in CH_2Cl_2 (0.5 mL) at room temperature was added 4-(dimethylamino)pyridine (2.0 mg), triethylamine (50 μl) and acetic anhydride (20 μl). The mixture was stirred for 6 h, and the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (petroleum ether/acetone 6:1, *v*/*v*) to afford compound **7c** (9 mg). $[\alpha]_D^{20.6}$ = −84.0 (*c* = 0.15, MeOH). UV (MeOH): 202 (4.41), 229 (4.57). IR (KBr): 3440, 3085, 2853, 1744, 1645, 1438, 1374, 1232, 1136, 1088, 1045, 956, 893, 674, 635, 602. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz): see *Table 3*. ESI-MS (pos.): 425 ([*M* + Na]$^+$). HR-ESI-MS (pos.): 425.2307 ([*M* + Na]$^+$, $C_{24}H_{34}NaO_5^+$; calc. 425.2304).

Methylation of the **7a**/**7b** (**7d**)

A solution of **7a**/**7b** (10 mg) in CH_2Cl_2 was treated with iodomethane (20 μl) and silver oxide (10.0 mg). The mixture was stirred at room temperature for 12 h, and the solvent was evaporated under reduced pressure. The residue was purified by HPLC (58% MeOH/H_2O) to provide **7d** (8 mg). $[\alpha]_D^{20.6}$ = −31.0 (*c* = 0.16, MeOH). UV (MeOH): 203 (3.54), 231 (3.70). IR (KBr): 3440, 2949, 2936, 2885, 2856, 1724, 1636, 1441, 1376, 1329, 1241, 1197, 1123, 1095, 1037, 955, 892, 581. ^{1}H-(500 MHz) and ^{13}C-NMR (125 MHz): see *Table 3*. ESI-MS (pos.): 397 ([*M* + Na]$^+$). HR-ESI-MS (pos.): 397.2346 ([*M* + Na]$^+$, $C_{23}H_{34}NaO_4^+$, calc. 397.2349).

PCC Oxidative of **7a**/**7b**

A solution of **7a**/**7b** (4.8 mg) in CH_2Cl_2 (0.5 ml) was oxidized with pyridinium chlorochromate (PCC) at room temperature for 1 h. Then, the solution was filtered, and the solvent was removed under reduced pressure. The residue was purified by HPLC (65% MeCN/H_2O) to provide **1** (2.3 mg) (*Scheme 2*). $[\alpha]_D^{20.6}$ = −38.3 (*c* = 0.16, MeOH). UV (MeOH): 231 (4.41). ECD (MeOH): Δε 207 + 12.7, Δε 294 − 2.33.

Crystallographic Data for **Hypofolin A** (**1**). $C_{22}H_{30}O_4$, *M* = 358.46, *a* = 6.07170(10) Å, *b* = 12.8248(2) Å, *c* = 25.9212(5) Å, α = 90°, β = 90°, γ = 90°, *V* = 2018.44(6) Å^3, *T* = 100(2) *K*, space group *P*212121, *Z* = 4, μ (CuK_α) = 0.637 mm^{-1}, 11,650 reflections measured, 3423 independent reflections (R_{int} = 0.0255). The final R_1 values were 0.0395 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1219 ($I > 2\sigma(I)$). The final R_1 values were 0.0404

(all data). The final $wR(F^2)$ values were 0.1231 (all data). The goodness of fit on F^2 was 1.096. *Flack* parameter = −0.06(4). Crystallographic data for **1** have been deposited at the *Cambridge Crystallographic Data Centre* under the reference number CCDC 1825715. Copies of the data can be obtained free of charge *via* www.ccdc.cam.ac.uk.

Cytotoxicity Assay

MTS (= 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) assays were used to measure the cytotoxicities of the isolated diterpenoids against HL-60, SMMC-7721, A-549, MCF-7, and SW-480 cell lines. Cells were seeded into 96-well plates at 5000 cells/well and incubated for 12 h. Then, different concentrations of isolated diterpenoids were added and incubated for 48 h. After removing the medium, 100 μl nutrient solution and 20 μl of MTS solution was added to each well and incubated for another 3 h, the *OD* value of each well was recorded at 492 nm. The IC_{50} value of each compound was calculated by the *Reed* and *Muench* method.

Nitric Oxide Production in RAW264.7 Macrophages

The murine macrophage cell line RAW264.7 was obtained from Cell Bank of the Chinese Academy of Sciences. RAW264.7 cells were seeded in 96-well cell culture plates and treated with serial dilutions of the compounds with a maximum concentration of 25 μM, followed by stimulation with 1 μg ml^{-1} LPS (*Sigma*) for 18 h. NO production in the supernatant was assessed by adding 100 μl of *Griess* reagent (Reagent *A* and Reagent *B*, respectively, *Sigma*). After 5 min of incubation, the absorbance at 570 nm was measured using a microplate reader (*Thermo*, *Bio-rad*, USA). NG-Monomethyl-L-arginine, monoacetate salt (L-NMMA, *Sigma*), a well-known nitric oxide synthase inhibitor, was used as a positive control. The viability of RAW264.7 cells was simultaneously evaluated using the MTS assay to exclude the interference of the cytotoxicity of the test compounds.

Supplementary Material

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbdv.201800124.

Acknowledgements

This work was financially supported by the *National Natural Science Foundation of China* (Nos. 21402212 and 81773611), and the *project from Guangxi Key Laboraratory of Functional Phytochemical Research and Utilization* (No. FPRU2015-1), the *State Key Laboratory of Phytochemistry and Plant Resources in West China* (No. P2015-ZZ16), and the *CAS 'Light of West China' Program and Youth Innovation Promotion Association CAS* (*X.-D. Wu*).

Author Contribution Statement

B. Cheng, *L.-F. Ding*, *T. Yang*, *Z.-Q. Xie*, and *L.-D. Song* collected plant samples, performed the experimental work, and wrote the manuscript; *X.-D. Wu* and *Q.-S. Zhao* supervised the entire project, revised, and polished the manuscript. All authors discussed the results and contributed to the final manuscript.

References

[1] B. Bavhure, M. Borive, J. Kadima, 'Haematic and Hepatroprotective Potentials of *Hypoestes triflora* Aqueous Leaf Extract in Guinea-Pigs', *Int. J. Pharm. Sci. Rev. Res.* **2014**, *9*, 3726 – 3732.

[2] A. Bhatt, Y. Naidoo, A. Nicholas, 'The Foliar Trichomes of *Hypoestes aristata* (Vahl) Sol. ex Roem. & Schult var aristata (Acanthaceae) a Widespread Medicinal Plant Species in Tropical Sub-Saharan Africa: With Comments on Its Possible Phylogenetic Significance', *Biol. Res.* **2010**, *43*, 403 – 409.

[3] A. S. Al-Hakimi, H. Maideen, A. Latiff, 'Pollen and Seed Morphology of *Rhinacanthus* Nees and *Hypoestes* Sol. ex R. Br. (Acanthaceae) of Yemen', *Sains Malaysiana* **2015**, *44*, 7 – 15.

[4] N. M. A. Musayeib, R. A. Mothana, G. A. Mohamed, S. R. M. Ibrahim, L. Maes, 'Hypoestenonols A and B, New Fusicoccane Diterpenes from *Hypoestes forskalei*', *Phytochem. Lett.* **2014**, *10*, 23 – 27.

[5] L. Rasoamiaranjanahary, A. Marston, D. Guilet, K. Schenk, F. Randimbivololona, K. Hostettmann, 'Antifungal Diterpenes from *Hypoestes serpens* (Acanthaceae)', *Phytochemistry* **2003**, *62*, 333 – 337.

[6] L. Rasoamiaranjanahary, D. Guilet, A. Marston, F. Randimbivololona, K. Hostettmann, 'Antifungal Isopimaranes from *Hypoestes serpens*', *Phytochemistry* **2003**, *64*, 543 – 548.

[7] E. A. Ojo-Amaize, E. J. Nchekwube, H. B. Cottam, O. A. Oyemade, A. A. Adesomoju, J. I. Okogun, '*Plasmodium berghei*: Antiparasitic Effects of Orally Administered Hypoestoxide in Mice', *Exp. Parasitol.* **2007**, *117*, 218 – 221.

[8] E. A. Ojo-Amaize, P. Kapahi, V. N. Kakkanaiah, T. Takahashi, T. Shalom-Barak, H. B. Cottam, A. A. Adesomoju, E. J. Nchekwube, O. A. Oyemade, M. Karin, J. I. Okogun, 'Hypoestoxide, a Novel Anti-inflammatory Natural Diterpene, Inhibits the Activity of IκB Kinase', *Cell. Immunol.* **2001**, *209*, 149 – 157.

[9] E. A. Ojo-Amaize, H. B. Cottam, O. A. Oyemade, J. I. Okogun, E. J. Nchekwube, 'Hypoestoxide Inhibits Tumor Growth in the Mouse CT26 Colon Tumor Model', *World J. Gastroenterol.* **2007**, *34*, 4586 – 4588.

[10] C. C. Shen, C. L. Ni, Y. L. Huang, R. L. Huang, C. C. Chen, 'Furanolabdane Diterpenes from *Hypoestes purpurea*', *J. Nat. Prod.* **2004**, *67*, 1947 – 1949.
[11] B. Andriamihaja, M. T. Martin, P. Rasoanaivo, F. Frappier, 'A New Diterpene from *Hypoestes serpens*', *J. Nat. Prod.* **2001**, *64*, 217 – 218.
[12] M. Shahid, A. Shahzad, T. Tripathi, F. Sobia, A. Sahai, A. Singh, A. Malik, F. Shujatullah, H. M. Khan, 'Recent Trends in Plant-Derived Antifungal Agents', *Anti-Infect. Agents Med. Chem.* **2009**, *8*, 36 – 49.
[13] I. Muhammad, J. S. Mossa, M. A. Al-Yahya, F. S. El-Feraly, A. T. Mcphae, 'Hypoestenone: A Fusicoccane Diterpene Ketone from *Hypoestes forskalei*', *Phytochemistry* **1997**, *44*, 125 – 129.
[14] I. Muhammad, J. S. Mossa, A. F. Ramadan, F. S. El-Feraly, C. D. Hufford, 'Additional Diterpene Ketones from *Hypoestes forskalei*', *Phytochemistry* **1998**, *47*, 1331 – 1336.
[15] A. A. Adesomoju, J. I. Okogu, 'Roseatoxide and Dihypoestoxidde: Additional New Diterpenoids from *Hypoestes rosea*', *J. Nat. Prod.* **1984**, *47*, 308 – 311.
[16] T. W. Starman, P. T. Gibson, 'Efficacy and Postharvest Persistence of Uniconazole Treatment on *Hypoestes phyllostachya*', *HortScience* **1992**, *27*, 819 – 820.
[17] X. D. Wu, D. Luo, W. C. Tu, Z. T. Deng, X. J. Chen, J. Su, X. Ji, Q. S. Zhao, 'Hypophyllins A–D, Labdane-Type Diterpenoids with Vasorelaxant Activity from *Hypoestes phyllostachya* 'Rosea'', *Org. Lett.* **2016**, *18*, 6484 – 6487.
[18] H. Tazaki, J. Zapp, H. Becker, 'Diterpenes from In Vitro Cultures of the Liverwort *Jamesoniella Autumnalis*', *Phytochemistry* **1995**, *39*, 859 – 868.

Received March 22, 2018
Accepted April 12, 2018

