Isoprenylated Chromone Derivatives from the Plant Endophytic Fungus *Pestalotiopsis fici*

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Pestaloficiols F−L (**1**−**7**), new isoprenylated chromone derivatives including one heterodimer (**7**), have been isolated from a scale-up fermentation extract of the plant endophytic fungus *Pestalotiopsis fici*. The structures of these compounds were elucidated primarily by NMR and MS methods. The absolute configurations of **1** and **4** were assigned using the modified Mosher method. Compounds **1**−**3**, **5**, and **6** displayed inhibitory effects on HIV-1 replication in C8166 cells, whereas **4**−**7** showed cytotoxic activity against the human tumor cell lines HeLa and MCF7.

Endophytic fungi inhabiting normal tissues of host plants are rich sources of bioactive natural products.[1−3] *Pestalotiopsis* spp. (Amphisphaeriaceae) are common in their distribution, and many are saprobes, while others are either pathogenic or endophytic to living plants.[4] Chemical studies of some species of this genus have afforded a variety of bioactive metabolites.[5−10] Our prior investigations of *P. fici* (AS 3.9138 = W106-1) grown in different solid-substrate fermentation cultures have resulted in the isolation of structurally diverse and biologically active metabolites, such as chloropupukeananin, the first chlorinated pupukeanane derivative with the unique tricyclo[4.3.1.$0^{3,7}$]decane skeleton and anti-HIV-1 effects,[11] and pestaloficiols A−E, new anti-HIV-1 cyclopropane derivatives isolated under different fermentation conditions.[12]

To identify the minor active components, the fungus *P. fici* was refermented in a larger scale using the solid fermentation culture in which chloropupukeananin was first isolated.[11] Fractionation of the crude extract afforded five new cyclohexanone derivatives including four heterodimers.[13] As a continuation of this work, seven new isoprenylated chromone derivatives (**1**−**7**), including a heterodimer (**7**), were obtained. Pestaloficiols F−L (**1**−**7**) were tested for anti-HIV-1 and cytotoxic activities. Details of the isolation, structure elucidation, and biological activities of these compounds are reported herein.

Results and Discussion

Pestaloficiol F (**1**) was obtained as a colorless oil. It was assigned the molecular formula $C_{16}H_{22}O_4$ (six degrees of unsaturation) on the basis of its HRESIMS (*m/z* 301.1412 $[M + Na]^+$; Δ −0.2 mmu). The ^1H and ^{13}C NMR spectra of **1** displayed resonances for one exchangeable proton, four methyl groups, three methylene units, two oxymethines, two oxygenated sp^3 quaternary carbons, two olefins, and one α,β-unsaturated ketone carbon (δ_C 190.5). These data accounted for all of the ^1H and ^{13}C NMR resonances and suggested that **1** was a tricyclic metabolite. A molecular formula search on $C_{16}H_{22}O_4$ identified the known compound A82775B (**8**), which has the same elemental composition as **1** and is also an isoprenylated chromone isolated from an unidentified fungus.[14] The ^1H and ^{13}C NMR spectra of **1** (Table 1) and **8** were nearly identical, except that the chemical shifts for some of the resonances corresponding to the polyoxygenated cyclohexane moiety were different. Analysis of the $^1H-^1H$ COSY and HMBC data of **1** established the same gross structure as **8**, indicating that **1** was a stereoisomer of **8** at one of the stereogenic carbons on the cylcohexane ring. This postulation was partially supported by the opposite signs of their specific rotations ($[\alpha]_D$ +293 for **1** vs −204 for **8**).

The relative configuration of **1** was determined by analysis of the $^1H-^1H$ coupling constants and NOESY data (Table 1). The large *trans*-diaxial-type coupling constant observed between H-5b and H-6 (11 Hz) indicated that they are pseudoaxially orientated, whereas a small coupling constant of 1.0 Hz between H-6 and H-7 places H-7 in a pseudoequatorial orientation of the cyclohexane ring. NOESY correlations of H-7 with H_2-9 and H-10 indicated that these protons are all on the same face of the cyclohexane ring, thereby establishing the relative configuration of **1**, which is different from **8** only at C-6.

The absolute configuration of **1** was assigned by application of the modified Mosher method.[15,16] Treatment of **1** with (*S*)-MTPA Cl and (*R*)-MTPA Cl afforded the (*R*)-MTPA ester (**1a**) and (*S*)-MTPA ester (**1b**), respectively. The difference in chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for the diastereomeric (*S*)-MTPA (**1b**) and (*R*)-MTPA (**1a**) esters was calculated to assign the 6*S* absolute configuration. Therefore, the 6*S*, 7*S*, and 8*S* absolute configuration was proposed for **1** on the basis of the $\Delta\delta$ results summarized in Figure 1.

Pestaloficiols G (**2**) and H (**3**) were obtained as an inseparable mixture of two isomers in a 4:1 ratio, as determined by integration of some well-resolved ^1H NMR resonances for each compound. Exhaustive efforts to separate this mixture were unsuccessful in even partially resolving them. Therefore, the structure elucidations of **2** and **3** were performed on the mixture. Compounds **2** and **3** were each assigned the molecular formula $C_{16}H_{22}O_4$ (six degrees of unsaturation) by its HRESIMS (*m/z* 301.1402 $[M + Na]^+$; Δ +0.8 mmu), which is the same as **1**. Analysis of the ^1H, ^{13}C, and HMQC NMR spectroscopic data of **2** and **3** revealed the same chromone core as found in **1**, except that the C-9 methylene (δ_H/δ_C 2.39; 2.54/47.3) and the C-8 sp^3 quaternary carbon (δ_C 59.7) were replaced by resonances for an olefin unit, and the olefinic proton H-10 was shifted downfield and observed as a doublet instead of a triplet in the spectra of **2** and **3**. The two exchangeable protons in each compound (δ_H 3.71 and 3.93 in **2**; 3.81 and 3.93 in **3**) were assigned as C-6−OH and C-7−OH, respectively, by relevant $^1H-^1H$ COSY correlations. Therefore, the gross structure of **2** and **3** was determined as shown.

The relative configurations of **2** and **3** were assigned by analysis of the $^1H-^1H$ coupling constants and NOESY data. The *trans*-diaxial-type coupling constant of 11 Hz between H-5b and H-6 and the small coupling constant of 2.0 Hz between H-6 and H-7 place H-6 and H-7 in pseudoaxial and pseudoequatorial orientations, respectively, of the cyclohexane ring. The C-8/C-9 olefin was

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Chart 1

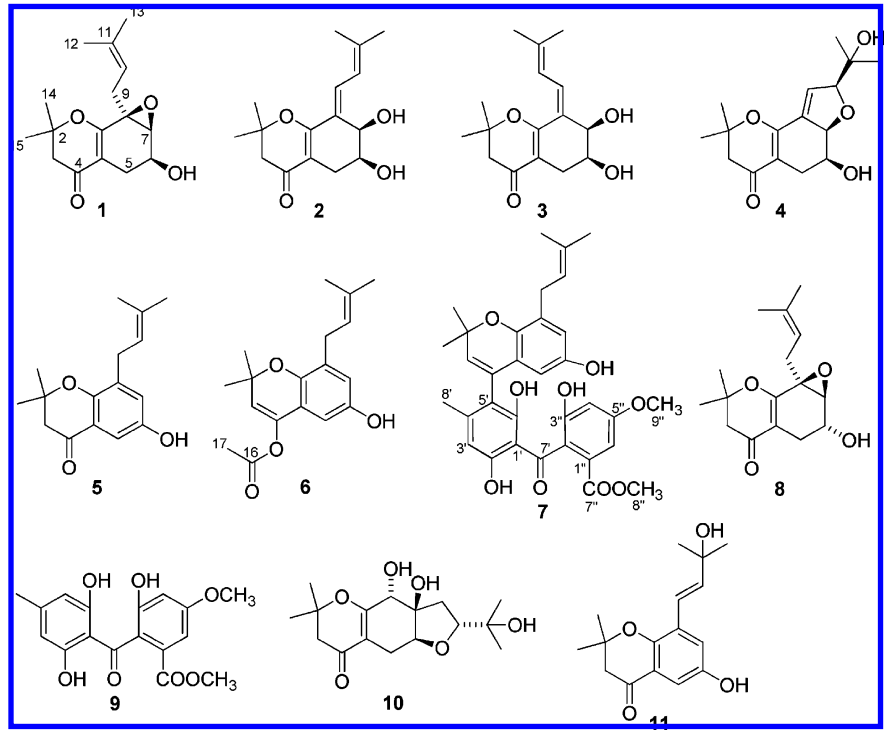

Table 1. NMR Spectroscopic Data (400 MHz, Acetone-d_6) for Pestaloficiol F (**1**)

position	δ_C, mult.	δ_H (J in Hz)	HMBC[a]	NOESY
2	81.8, qC			
3a	47.3, CH_2	2.54, d (16)	2, 4, 14, 15	
3b		2.39, d (16)	2, 4, 4a, 14, 15	
4	190.5, qC			
4a	108.6, qC			
5a	25.0, CH_2	2.75, dd (16, 6.5)	4, 4a, 6, 7, 8a	
5b		1.81, dd (16, 11)	4, 4a, 6, 7, 8a	
6	67.1, CH	4.05, dddd (11, 6.5, 6.0, 1.0)	5, 7	
7	64.8, CH	3.45, d (1.0)	5, 6, 8, 8a, 9	9, 10
8	59.7, qC			
8a	165.0, qC			
9a	29.5, CH_2	2.78, dd (15, 6.5)	7, 8, 8a, 10, 11	7
9b		2.26, dd (15, 6.5)	7, 8, 8a, 10, 11	7
10	119.0, CH	5.15, t (6.5)	9, 12, 13	7
11	135.0, qC			
12	18.2, CH_3	1.67, s	10, 11, 13	
13	26.0, CH_3	1.71, s	10, 11, 12	
14	24.8, CH_3	1.37, s	2, 3, 15	
15	27.2, CH_3	1.46, s	2, 3, 14	
OH-6		4.34, d (6.0)		

[a] HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon.

assigned *E*-geometry on the basis of NOESY correlation of H-7 with H-10 in the major isomer (**2**), whereas in the minor one (**3**), it was assigned *Z*-geometry by NOESY correlation of H-7 with H-9. Due to sample limitation, and the fact that we were unable to separate these isomers, the absolute configurations of **2** and **3** were not assigned by chemical derivatizations. However, from biogenetic considerations, C-6 and C-7 in both **2** and **3** presumably have the same absolute configurations as in **1**.

Pestaloficiol I (**4**) gave a pseudomolecular ion $[M + Na]^+$ peak at m/z 317.1362 (Δ −0.3 mmu) by HRESIMS, consistent with a molecular formula of $C_{16}H_{22}O_5$ (six degrees of unsaturation). Analysis of its ^1H, ^{13}C, and HMQC NMR data revealed the same dihydropyranone ring as found in **1**−**3**, but the signals for the isoprenyl group attached cyclohexane moiety were significantly different. The C-5−C-7 (including OH-6) and C-9−C-10 fragments were identified in **4** by $^1H-^1H$ COSY data. HMBC correlations from H-6 to C-4a and C-8 and from H-9 to C-7, C-8a, and C-10 completed the cyclohexane moiety with the C-8/C-9 olefin attached to C-8. Those from H_3-12 and H_3-13 to C-10 and C-11 led to the connection of C-10, C-12, and C-13 to C-11. A correlation from H-7 to C-10 established the dihydrofuran ring. The only remaining exchangeable proton (δ_H 4.13) was assigned as the OH group attached to the sp^3 quaternary carbon C-11 (δ_C 72.1) on the basis of an HMBC cross-peak from OH-11 to C-12. On the basis of these data, the gross structure of **4** was established as shown.

The relative configuration of **4** was determined by analysis of the $^1H-^1H$ coupling constants and NOESY data (Figure 2). The $^1H-^1H$ coupling patterns for H_2-5, H-6, and H-7 indicated that H-6 is pseudoequatorially oriented. The orientations of H-5b and H-7 were also assigned as pseudoaxial by NOESY correlations observed for these two protons. Correlations of H-6 with H_3-12 revealed their proximity in space, thereby completing the relative configuration of **4** as shown. The absolute configuration of **4** was assigned using the modified Mosher method. The difference in chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for the diastereomeric esters **4b** and **4a** was calculated to assign the 6*S* absolute configuration. Therefore, the 6*S*, 7*R*, and 10*S* absolute configuration was proposed for **4** on the basis of the $\Delta\delta$ results summarized in Figure 3.

The molecular formula of pestaloficiol J (**5**) was assigned as $C_{16}H_{20}O_3$ (seven degrees of unsaturation) by its HRESIMS (m/z 283.1307 $[M + Na]^+$; Δ −0.2 mmu), 18 mass units less than **1**.

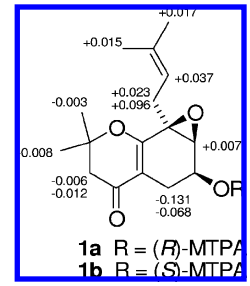

Figure 1. $\Delta\delta$ values (in ppm) = $\delta_S - \delta_R$ obtained for (*S*)- and (*R*)-MTPA esters **1b** and **1a**.

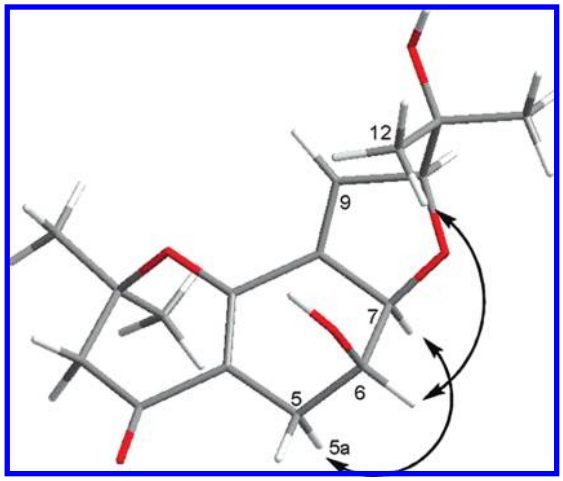

Figure 2. Key NOESY correlations for pestaloficiol I (**4**).

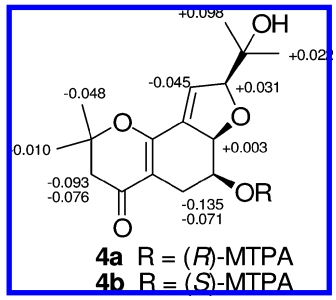

Figure 3. $\Delta\delta$ values (in ppm) $= \delta_S - \delta_R$ obtained for (*S*)- and (*R*)-MTPA esters **4b** and **4a**.

NMR spectra of **5** displayed resonances characteristic of an aryl ring, implying that the cyclohexane ring present in **1**−**3** was aromatized. This observation was supported by HMBC correlations from H-5 to C-4, C-7, and C-8a and from H-7 to C-5, C-8a, and C-9. Therefore, the gross structure of **5** was assigned as shown.

Pestaloficiol K (**6**) gave a pseudomolecular ion $[M + Na]^+$ peak at *m*/*z* 325.1420 (Δ −1.0 mmu) by HRESIMS ($C_{18}H_{22}O_4$, eight degrees of unsaturation), 42 mass units higher than **5**. The NMR data of **6** revealed structural features similar to those found in **5**, except that the resonances for C-3 (δ_C 49.2) and C-4 (δ_C 192.6) were replaced by those for an olefin (δ_C 118.8 and 143.1, respectively), suggesting that the ketone functionality in **5** was tautomerized to the enol form in **6**. NMR resonances for an acetyl group (δ_H 2.24; δ_C 20.7 and 168.8) were also observed, indicating that the C-4 (δ_C 143.1) oxygen of **6** was acylated. These observations were supported by HMBC correlations from the olefinic proton H-3 to C-2, C-4, C-4a, C-14, and C-15.

The elemental composition of pestaloficiol L (**7**) was established as $C_{33}H_{34}O_9$ (17 degrees of unsaturation) by its HRESIMS (*m*/*z* 597.2093 $[M + Na]^+$; Δ +0.2 mmu). Interpretation of its ^1H, ^{13}C, and HMQC NMR spectroscopic data (Table 2) revealed the presence of four exchangeable protons, seven methyl groups (two *O*-methyls), one methylene unit, one oxygenated sp^3 quaternary carbon, 22 olefinic/aromatic carbons, one carboxyl (δ_C 166.9), and one ketone carbon (δ_C 200.9). These NMR data are characteristic of **6** (excluding the acetyl group) and the known compound isosulochrin (**9**), which was also isolated from the same crude extract in this work,[13,17] suggesting that **7** could be a heterodimeric metabolite derived from **6** and **9**. In addition, the molecular formula of **7** ($C_{33}H_{34}O_9$) is equivalent to the summation of those for the deacetyl analogue of **6** ($C_{16}H_{20}O_3$) and **9** ($C_{17}H_{16}O_7$) subtracting one H_2O molecule. This postulation was supported by relevant HMBC data, which were in agreement with the presence of the partial structures for **6** and **9**. An HMBC correlation from H-3 to C-5′ connected the two subunits through the C-3−C-5′ bond, completing the gross structure of **7** as depicted.

Table 2. NMR Spectroscopic Data (400 MHz, Acetone-d_6) for Pestaloficiol L (**7**)

position	δ_C, mult.	δ_H (*J* in Hz)	HMBC[a]
2	76.0, qC		
3	132.9, CH	5.57, s	2, 4, 4a, 5′,14, 15
4	123.6, qC		
4a	129.9, qC		
5	109.4, CH	6.01, d (3.0)	4a, 6, 7, 8a
6	151.4, qC		
7	116.6, CH	6.49, d (3.0)	5, 6, 8a, 9
8	130.4, qC		
8a	144.4, qC		
9	29.0, CH_2	3.23, d (7.0)	7, 8, 8a, 10, 11
10	124.0, CH	5.29, t (7.0)	8, 9, 12, 13
11	131.9, qC		
12	17.9, CH_3	1.73, s	10, 11, 13
13	25.9, CH_3	1.71, s	10, 11, 12
14	27.4, CH_3	1.44, s	2, 3, 15
15	27.8, CH_3	1.47, s	2, 3, 14
1′	110.6, qC		
2′	160.6, qC		
3′	108.7, CH	6.29, s	1′, 2′, 5′, 8′
4′	147.0, qC		
5′	117.6, qC		
6′	161.3, qC		
7′	200.9, qC		
8′	20.5, CH_3	2.07, s	3′, 4′, 5′
1″	130.5, qC		
2″	127.3, qC		
3″	155.9, qC		
4″	106.4, CH	6.65, d (2.0)	2″, 3″, 5″, 6″
5″	160.9, qC		
6″	106.4, CH	6.97, d (2.0)	1″, 2″, 4″, 5″, 7″
7″	166.9, qC		
8″	52.3, CH_3	3.68, s	7″
9″	56.0, CH_3	3.80, s	5″
OH-6		7.40, br s	
OH-2′		11.4, br s	
OH-6′		9.52, br s	
OH-3″		9.52, br s	

[a] HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon.

Table 3. Biological Activities of Compounds **1**−**7**

		cytotoxicity (IC_{50}; μM)	
compound	HIV-1 (EC_{50}; μM)	HeLa	MCF7
1	>100	>143.9	>143.9
2 and **3**	89.2	>143.9	>143.9
4	>100	>136.1	136.1
5	8.0	21.2	>153.8
6	78.2	99.3	>132.5
7	>100	8.7	17.4
indinavir sulfate	0.0082		
5-fluorouracil		10.0	15.0

Compounds **1**−**7** were evaluated for anti-HIV-1 and cytotoxic activities (Table 3). Compound **5** showed an inhibitory effect on HIV-1 replication in C8166 cells, with an EC_{50} value of 8.0 μM (the CC_{50} value is greater than 100 μM; the positive control indinavir sulfate showed an EC_{50} value of 8.2 nM). Compound **7** displayed cytotoxic activity against the HeLa and MCF7 cells, with IC_{50} values of 8.7 and 17.4 μM, respectively (the positive control 5-fluorouracil showed IC_{50} values of 10.0 and 15.0 μM, respectively).

Pestaloficiols F−L (**1**−**7**) are new isoprenylated chromone derivatives. Compound **1** is a C-6 stereoisomer of A82775B (**8**),[14] whereas **2** and **3** were isolated as a mixture of the *E*/*Z* isomers that could originate from **1** via ring-opening of the C-7−C-8 epoxide followed by dehydration of the isoprenyl group. Compound **4** is structurally related to pestalotheol A (**10**),[18] but differs in having

a different fusion pattern between the cyclohexane and the furan rings. Compounds **5** and **6** are new analogues of the known compound **11**,[19] whereas the heterodimer **7** could be derived from **6** and isosulochrin (**9**).[13,17] Compounds **4**−**6** could be derived from **1**−**3** via reactions including oxidation, reduction, and cyclization. The discovery of these new bioactive metabolites further expanded the structural diversity of the bioactive products produced by the fungus *P. fici*.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ^{1}H and ^{13}C NMR data were acquired with Varian Mercury-400 and -500 spectrometers using solvent signals (acetone-d_6: δ_H 2.05/δ_C 29.8, 206.1; pyridine-d_5: δ_H 7.21, 7.58, 8.73) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS data were recorded on a Bruker Esquire 3000^{plus} spectrometer, and HRESIMS data were obtained using Bruker APEX III 7.0 T and APEXII FT-ICR spectrometers, respectively.

Fungal Material. The culture of *P. fici* was isolated from branches of *Camellia sinensis* in the suburb of Hangzhou, in April 2005. The isolate was identified as *P. fici* by one of authors (L.G.) based on sequence (GenBank Accession number DQ812914) analysis of the ITS region of the rDNA and assigned the accession number AS 3.9138 (= W106-1) in China General Microbial Culture Collection (CGMCC) at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 10 days. Agar plugs were used to inoculate 250 mL Erlenmeyer flasks, each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract), and the final pH of the media was adjusted to 6.5 before sterilization. Flask cultures were incubated at 25 °C on a rotary shaker at 170 rpm for 5 days. Fermentation was carried out in twelve 500 mL Erlenmeyer flasks each containing 80 g of rice. Spore inoculum was prepared by suspension in sterile, distilled H_2O to give a final spore/cell suspension of 1×10^6/mL. Distilled H_2O (100 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 lb/in.^{2} for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 days.

Extraction and Isolation. The fermented material was extracted with EtOAc (4 × 1.0 L), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (10 0.0 g), which was fractionated by silica gel vacuum liquid chromatography (VLC) using petroleum ether−EtOAc gradient elution. The fraction (136 mg) eluted with 32% EtOAc was separated by Sephadex LH-20 column chromatography (CC) eluting with 1:1 $CHCl_3$−CH_3OH. The resulting subfractions were combined and further purified by semipreparative RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 45% CH_3OH in H_2O for 2 min, followed by 45− 60% for 28 min; 2 mL/min) to afford a mixture of **2** and **3** (4.3 mg). The fraction eluted with 8% EtOAc (50 mg) was purified by HPLC (70% CH_3OH in H_2O for 2 min, followed by 70−75% for 25 min; 2 mL/min) to afford **1** (4.0 mg). Fractions eluted with 10−33% EtOAc were combined (480 mg) and fractionated again by Sephadex LH-20 CC using $CHCl_3$−CH_3OH (1:1). Purification of resulting subfractions with different gradients afforded pestaloficiols I (**4**; 2.4 mg; 70% CH_3OH in H_2O for 2 min, followed by 70−79% for 25 min), J (**5**; 3.0 mg; 60% CH_3OH in H_2O for 2 min, followed by 60−90% for 23 min), K (**6**; 2.0 mg; 70% CH_3OH in H_2O for 2 min, followed by 70−89% for 20 min), and L (**7**; 1.4 mg; 45% CH_3OH in H_2O for 2 min, followed by 45−60% for 28 min).

Pestaloficiol F (1): colorless oil; $[\alpha]^{25}_D$ +293 (*c* 0.1, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 278 (4.11) nm; IR (neat) ν_{max} 3407 (br), 2977, 2925, 1660, 1609, 1403, 1250, 1167 cm^{-1}; ^{1}H, ^{13}C NMR, HMBC, and NOESY data see Table 1; HRESIMS *m/z* 301.1412 (calcd for $C_{16}H_{22}O_4Na$, 301.1410).

Preparation of (*R*)-MTPA Ester (1a) and (*S*)-MTPA Ester (1b). A sample of **1** (1.0 mg, 0.004 mmol), (*S*)-MTPA Cl (2.0 μL, 0.011 mmol), and pyridine-d_5 (0.5 mL) were allowed to react in an NMR tube at ambient temperature for 24 h. The ^{1}H NMR data of the (*R*)-MTPA ester derivative (**1a**) were obtained directly on the reaction mixture: ^{1}H NMR (pyridine-d_5, 400 MHz) δ 5.77 (1H, ddd, J = 11, 7.2, 2.0 Hz, H-6), 5.22 (1H, t, J = 6.5 Hz, H-10), 3.52 (1H, d, J = 2.0 Hz, H-7), 2.87 (1H, dd, J = 15, 11 Hz, H-5a), 2.80 (1H, dd, J = 16, 6.5 Hz, H-9a), 2.55 (1H, d, J = 17 Hz, H-3a), 2.50 (1H, d, J = 17 Hz, H-3b), 2.43 (1H, dd, J = 15, 11 Hz, H-5b), 2.42 (1H, dd, J = 16, 6.5 Hz, H-9b), 1.63 (3H, s, H_3-13), 1.58 (3H, s, H_3-12), 1.31 (3H, s, H_3-15), 1.28 (3H, s, H_3-14).

Another sample of **1** (1.0 mg, 0.004 mmol), (*R*)-MTPA Cl (2.0 μL, 0.011 mmol), and pyridine-d_5 (0.5 mL) was processed as described above for **1a** to afford **1b**: ^{1}H NMR (pyridine-d_5, 400 MHz) δ 5.79 (1H, ddd, J = 11, 7.2, 2.0 Hz, H-6), 5.26 (1H, t, J = 6.5 Hz, H-10), 3.53 (1H, d, J = 2.0 Hz, H-7), 2.90 (1H, dd, J = 16, 6.5 Hz, H-9a), 2.80 (1H, dd, J = 15, 11 Hz, H-5a), 2.53 (1H, d, J = 17 Hz, H-3a), 2.49 (1H, d, J = 17 Hz, H-3b), 2.44 (1H, dd, J = 16, 6.5 Hz, H-9b), 2.30 (1H, dd, J = 15, 11 Hz, H-5b), 1.64 (3H, s, H_3-13), 1.60 (3H, s, H_3-12), 1.30 (3H, s, H_3-15), 1.27 (3H, s, H_3-14).

Pestaloficiols G and H (2 and 3): pale yellow oil; $[\alpha]^{25}_D$ +136 (*c* 0.1, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 340 (4.09) nm; IR (neat) ν_{max} 3383, 2975, 2929, 1627, 1583, 1418, 1370, 1247, 1152 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) **2**: δ 7.22 (1H, d, J = 12 Hz, H-9), 6.41 (1H, d, J = 12 Hz, H-10), 4.77 (1H, br d, J = 2.0 Hz, H-7), 3.93 (1H, br s, OH-7), 3.71 (1H, br s, OH-6), 3.62 (1H, ddd, J = 11, 5.4, 2.0 Hz, H-6), 2.69 (1H, dd, J = 16, 5.4 Hz, H-5a), 2.51 (1H, d, J = 16 Hz, H-3a), 2.42 (1H, d, J = 16 Hz, H-3b), 2.26 (1H, dd, J = 16, 11 Hz, H-5b), 1.91 (3H, s, H_3-13), 1.88 (3H, s, H_3-12), 1.43 (3H, s, H_3-15), 1.39 (3H, s, H_3-14); **3**: δ 7.08 (1H, d, J = 12 Hz, H-10), 6.74 (1H, d, J = 12 Hz, H-9), 4.26 (1H, br d, J = 1.6 Hz, H-7), 3.93 (1H, br s, OH-7), 3.81 (1H, br s, OH-6), 3.80 (1H, ddd, J = 11, 5.4, 1.6 Hz, H-6), 2.69 (1H, dd, J = 16, 5.4 Hz, H-5a), 2.50 (1H, d, J = 16 Hz, H-3a), 2.45 (1H, d, J = 16 Hz, H-3b), 2.26 (1H, dd, J = 16, 11 Hz, H-5b), 1.87 (3H, s, H_3-13), 1.84 (3H, s, H_3-12), 1.46 (3H, s, H_3-15), 1.44 (3H, s, H_3-14); ^{13}C NMR (acetone-d_6, 100 MHz) **2**: δ 191.4 (C, C-4), 160.6 (C, C-8a), 144.6 (C, C-11), 130.8 (C, C-8), 130.0 (CH, C-9), 121.8 (CH, C-10), 109.4 (C, C-4a), 79.8 (C, C-2), 69.6 (CH, C-6), 67.8 (CH, C-7), 47.9 (CH_2, C-3), 27.2 (CH_3, C-15), 26.7 (CH_3, C-13), 25.4 (CH_3, C-14), 24.8 (CH_2, C-5), 18.7 (CH_3, C-12); **3**: δ 191.8 (C, C-4), 162.1 (C, C-8a), 143.2 (C, C-11), 133.3 (CH, C-9), 129.1 (C, C-8), 124.5 (CH, C-10), 110.3 (C, C-4a), 80.3 (C, C-2), 76.4 (CH, C-7), 69.6 (CH, C-6), 47.8 (CH_2, C-3), 27.0 (CH_3, C-13), 26.5 (CH_3, C-15), 26.1 (CH_2, C-5), 25.4 (CH_3, C-14), 17.9 (CH_3, C-12); HMBC data (acetone-d_6, 400 MHz) H_2-3 → C-2, 4, 4a, 14, 15; H_2-5 → C-4, 4a, 6, 7, 8a; H-7 → C-5, 6, 8, 8a, 9; H-9 → C-7, 8, 8a, 10, 11; H-10 → C-8, 9, 12, 13; H_3-12 → C-10, 11, 13; H_3-13 → C-10, 11, 12; H_3-14 → C-2, 3, 15; H_3-15 → C-2, 3, 14; NOESY correlations (acetone-d_6, 500 MHz) **2**: H-7 → H-10; H-10 → H-7; **3**: H-7 → H-9; H-9 → H-7; HRESIMS *m/z* 301.1402 (calcd for $C_{16}H_{22}O_4Na$, 301.1410).

Pestaloficiol I (4): pale yellow oil; $[\alpha]^{25}_D$ −79 (*c* 0.1, CH_3 OH); UV (CH_3OH) λ_{max} (log ε) 304 (4.04) nm; IR (neat) ν_{max} 3378 (br), 2975, 2932, 1643, 1574, 1424, 1165 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ 6.36 (1H, d, J = 1.5 Hz, H-9), 4.86 (1H, dd, J = 4.0, 3.3 Hz, H-7), 4.76 (1H, dd, J = 4.0, 1.5 Hz, H-10), 4.35 (1H, br s, OH-6), 4.19 (1H, ddd, J = 3.3, 3.0, 2.5 Hz, H-6), 4.13 (1H, s, OH-11), 2.71 (1H, dd, J = 18, 2.5 Hz, H-5a), 2.57 (1H, d, J = 17 Hz, H-3a), 2.44 (1H, d, J = 17 Hz, H-3b), 2.32 (1H, dd, J = 18, 3.0 Hz, H-5b), 1.44 (3H, s, H_3-15), 1.38 (3H, s, H_3-14), 1.23 (6H, s, H_3-12/H_3-13); ^{13}C NMR (acetone-d_6, 100 MHz) δ 192.3 (C, C-4), 157.0 (C, C-8a), 135.0 (C, C-8), 128.5 (CH, C-9), 109.2 (C, C-4a), 94.6 (CH, C-10), 80.9 (C, C-2), 86.6 (CH, C-7), 72.1 (C, C-11), 66.8 (CH, C-6), 48.1 (CH_2, C-3), 29.6 (CH_2, C-5), 27.4 (CH_3, C-13), 27.0 (CH_3, C-15), 26.4 (CH_3, C-12), 25.5 (CH_3, C-14); HMBC data (acetone-d_6, 400 MHz) H_2-3 → C-2, 4, 4a, 14, 15; H_2-5 → C-4, 4a, 6, 7, 8a; H-6 → C-4a, 7, 8; H-7 → C-10; H-9 → C-7, 8a, 10; H-10 → C-8, 9, 12, 13; H_3-12 → C-10, 11, 13; H_3-13 → C-10, 11, 12; H_3-14 → C-2, 3, 15; H_3-15 → C-2, 3, 14; OH-11 → C-12; NOESY correlations (acetone-d_6, 500 MHz) H-5a → H-7; H-7 → H-5a; H-6 → H_3-12; H_3-12 → H-6; HRESIMS *m/z* 317.1362 (calcd for $C_{16}H_{22}O_5Na$, 317.1359).

Preparation of (*R*)-MTPA Ester (4a) and (*S*)-MTPA Ester (4b). A sample of **4** (1.0 mg, 0.003 mmol), (*S*)-MTPA Cl (2.0 μL, 0.011 mmol), and pyridine-d_5 (0.5 mL) were allowed to react in an NMR tube at ambient temperature for 24 h, with the ^{1}H NMR data of the (*R*)-MTPA ester derivative (**4a**) obtained directly on the reaction mixture: ^{1}H NMR (pyridine-d_5, 400 MHz) δ 6.92 (1H, d, J = 1.5 Hz, H-9), 5.92 (1H, ddd, J = 3.3, 3.0, 2.5 Hz, H-6), 5.09 (1H, dd, J = 4.0, 3.3 Hz, H-7), 4.96 (1H, dd, J = 4.0, 1.5 Hz, H-10), 2.78 (1H, dd, J = 18, 2.5 Hz, H-5a), 2.77 (1H, d, J = 17 Hz, H-3a), 2.74 (1H, d, J = 17 Hz, H-3b), 2.63 (1H, dd, J = 18, 3.0 Hz, H-5b), 1.44 (3H, s, H_3-15), 1.35 (3H, s, H_3-14), 1.32 (3H, s, H_3-13), 1.09 (3H, s, H_3-12).

Similarly, the reaction mixture from another sample of **4** (1.0 mg, 0.003 mmol), (*R*)-MTPA Cl (2.0 μL, 0.011 mmol), and pyridine-d_5 (0.5 mL) was processed as described above for **4a** to afford **4b**: ^{1}H NMR (pyridine-d_5, 400 MHz) δ 6.87 (1H, d, J = 1.5 Hz, H-9), 5.91 (1H, ddd, J = 3.3, 3.0, 2.5 Hz, H-6), 5.10 (1H, dd, J = 4.0, 3.3 Hz, H-7), 4.99 (1H, dd, J = 4.0, 1.5 Hz, H-10), 2.71 (1H, dd, J = 18, 2.5 Hz, H-5a), 2.69 (1H, d, J = 17 Hz, H-3a), 2.65 (1H, d, J = 17 Hz, H-3b), 2.49 (1H, dd, J = 18, 3.0 Hz, H-5b), 1.45 (3H, s, H_3-15), 1.40 (3H, s, H_3-14), 1.35 (3H, s, H_3-13), 1.12 (3H, s, H_3-12).

Pestaloficiol J (5): colorless oil; UV (CH_3OH) λ_{max} (log ε) 214 (3.97), 251 (3.67) nm; IR (neat) ν_{max} 3401 (br), 2977, 2924, 1669, 1614, 1469, 1250 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.07 (1H, br s, OH-6), 7.05 (1H, d, J = 2.5 Hz, H-5), 6.92 (1H, d, J = 2.5 Hz, H-7), 5.26 (1H, t, J = 7.5 Hz, H-10), 3.26 (2H, d, J = 7.5 Hz, H_2-9), 2.67 (2H, s, H_2-3), 1.71 (6H, s, H_3-12/H_3-13), 1.41 (6H, s, H_3-14/H_3-15); ^{13}C NMR (acetone-d_6, 100 MHz) δ 192.6 (C, C-4), 152.1 (C, C-8a), 151.5 (C, C-6), 133.2 (C, C-8), 133.1 (C, C-11), 124.7 (CH, C-7), 123.0 (CH, C-10), 121.3 (C, C-4a), 108.4 (CH, C-5), 79.3 (C, C-2), 49.2 (CH_2, C-3), 28.8 (CH_2, C-9), 26.6 (CH_3, C-14/C-15), 25.8 (CH_3, C-13), 17.9 (CH_3, C-12); HMBC data (acetone-d_6, 400 MHz) H_2-3 → C-2, 4, 4a, 14, 15; H-5 → C-4, 7, 8a; H-7 → C-5, 8a, 9; H_2-9 → C-7, 8, 8a, 10, 11; H-10 → C- 9, 12, 13; H_3-12 → C-10, 11, 13; H_3-13 → C-10, 11, 12; H_3-14 → C-2, 3, 15; H_3-15 → C-2, 3, 14; HRESIMS *m*/*z* 283.1307 (calcd for $C_{16}H_{20}O_3Na$, 283.1305).

Pestaloficiol K (6): colorless oil; UV (CH_3OH) λ_{max} (log ε) 215 (4.04), 255 (3.79) nm; IR (neat) ν_{max} 3357 (br), 2974, 2929, 1766, 1667, 1595, 1451, 1365, 1195 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.77 (1H, br s, OH-6), 6.56 (1H, d, J = 2.5 Hz, H-7), 6.43 (1H, d, J = 2.5 Hz, H-5), 5.47 (1H, s, H-3), 5.23 (1H, t, J = 7.5 Hz, H-10), 3.26 (2H, d, J = 7.5 Hz, H_2-9), 2.24 (3H, s, H_3-17), 1.69 (3H, s, H_3-12), 1.71 (3H, s, H_3-13), 1.42 (6H, s, H_3-14/H_3-15); ^{13}C NMR (acetone-d_6, 100 MHz) δ 168.9 (C, C-16), 151.6 (C, C-6), 146.2 (C, C-8a), 143.1 (C, C-4), 132.3 (C, C-11), 131.1 (C, C-8), 123.5 (CH, C-10), 119.8 (C, C-4a), 118.8 (CH, C-3), 117.7 (CH, C-7), 106.5 (CH, C-5), 77.1 (C, C-2), 28.8 (CH_2, C-9), 28.0 (CH_3, C-14/C-15), 25.8 (CH_3, C-13), 20.7 (CH_3, C-17), 17.9 (CH_3, C-12); HMBC data (acetone-d_6, 400 MHz) H-3 → C-2, 4, 4a, 14, 15; H-5 → C-7, 8a; H-7 → C-5, 8a; H_2-9 → C-7, 8, 8a, 10, 11; H-10 → C- 9, 12, 13; H_3-12 → C-10, 11, 13; H_3-13 → C-10, 11, 12; H_3-14 → C-2, 3, 15; H_3-15 → C-2, 3, 14; H_3-17 → C-16; OH-6 → C-5, 6, 7; HRESIMS *m*/*z* 325.1420 (calcd for $C_{18}H_{22}O_4Na$, 325.1410).

Pestaloficiol L (7): pale yellow oil; UV (CH_3OH) λ_{max} (log ε) 215 (4.39), 255 (4.25) nm; IR (neat) ν_{max} 3374, 2924, 2854, 1717, 1606, 1445, 1241 cm^{-1}; ^{1}H, ^{13}C NMR and HMBC data see Table 2; HRESIMS *m*/*z* 597.2093 (calcd for $C_{33}H_{34}O_9Na$, 597.2095).

Anti-HIV Bioassays. Anti-HIV assays included cytotoxicity and HIV-1 replication inhibition evaluations. Cytotoxicity was measured by the MTT method as described in the literature.[12] Cells (3×10^4/well) were seeded into a 96-well microtiter plate in the absence or presence of various concentrations of test compounds in triplicate and incubated at 37 °C in a humid atmosphere of 5% CO_2. After a 4-day incubation, cell viability was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. The concentration that caused the reduction of viable cells by 50% (CC_{50}) was determined. In parallel with the MTT assay, a HIV-1 replication inhibition assay was determined by p24 antigen capture ELISA. C8166 cells were exposed to HIV-1 (MOI = 0.058) at 37 °C for 1.5 h, washed with PBS to remove free viruses, and then seeded into a 96-well microtiter plate at 3×10^4 cells per well in the absence or presence of test compounds (indinavir sulfate was used as positive control). After 4 days, the supernatant was collected and inactivated by 0.5% Triton X-100. The supernatant was diluted three times, added to the plate coating with anti-p24 McAb (provided by Dr. Bin Yan, Wuhan Institute of Virology, Wuhan, People's Republic of China), and incubated at 37 °C for 1 h. After washing five times with PBST, the HRP-labeled anti-p24 antibody (provided by Dr. Bin Yan) was added and incubated at 37 °C for 1 h. The plate was washed five times with PBST, followed by adding OPD reaction mixture. The assay plate was read at 490 nm using a microplate reader within 30 min. The inhibition rate and the EC_{50} based on p24 antigen expression level were calculated.

MTT Assay.[20] In 96-well plates, each well was plated with 10^4 cells. After cell attachment overnight, the medium was removed, and each well was treated with 50 μL of medium containing 0.2% DMSO or appropriate concentrations of test compounds (10 mg/mL as stock solution of a compound in DMSO and serial dilutions). Cells were treated at 37 °C for 4 h in a humidified incubator at 5% CO_2 first and then were allowed to grow for another 48 h after the medium was changed to fresh Dulbecco's modified Eagle medium (DMEM). MTT (Sigma) was dissolved in serum-free medium or PBS at 0.5 mg/mL and sonicated briefly. In the dark, 50 μL of MTT/medium was added into each well after the medium was removed from wells and incubated at 37 °C for 3 h. Upon removal of MTT/medium, 100 μL of DMSO was added to each well, which were agitated at 60 rpm for 5 min to dissolve the precipitate. The assay plate was read at 540 nm using a microplate reader.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of pestaloficiols F−L (**1**−**7**). This material is available free of charge via the Internet at http://pubs.acs.org.

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