FULL PAPER

New Cytotoxic Meroterpenoids from the Plant Endophytic Fungus *Pestalotiopsis fici*

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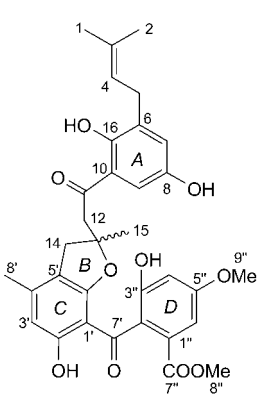
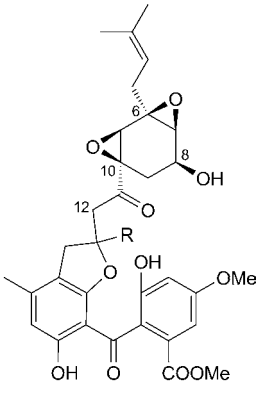
Pestalofones I – K (**1** – **3**), three new dimeric meroterpenoids with 2-(7-benzoyl-2,3-dihydrobenzofuran-2-yl)-1-phenylethan-1-one (in **1**) and 2-(7-benzoyl-2,3-dihydrobenzofuran-2-yl)-1-(3,8-dioxatricyclo[5.1.0.0^{2,4}]oct-4-yl)ethan-1-one (in **2** and **3**) skeletons, were isolated from the solid cultures of the plant endophytic fungus *Pestalotiopsis fici*. The structures of **1** – **3** were elucidated by NMR experiments. Compound **1** was found to be present as a racemic mixture. The absolute configurations of **2** and **3** were deduced by analogy to the previously isolated metabolites pestalofones G and H (**4** and **5**) from the same fungus. Biogenetically, compounds **1** – **3** are derived from the same precursors (co-isolated compounds **9** and **10**) as the previously isolated compounds **4** – **8**. Compounds **2** and **3** showed weak cytotoxic activities against four human tumor cell lines T24, HeLa, A549, and MCF-7.

Introduction. – Meroterpenoids, a characteristic type of secondary metabolites, represent merged polyketide-terpenoid structures. Fungi are known as producers of meroterpenoids with novel structures and diverse bioactivities. Examples include applanatumin A, a novel meroterpenoid dimer with potent antifibrotic activity from *Ganoderma applanatum* [1], albatrelins A – C, three novel dimers with cytotoxicity from *Albatrelleus ovinus* [2], and yaminterritrems A and B with a novel skeleton and inhibition of cyclooxygenase-2 expression from *Aspergillus terreus* [3]. Endophytic fungi inhabiting normal tissues of hosts without causing apparent symptoms of pathogenesis are rich sources of bioactive natural products [4 – 6]. As one class of the most widely distributed endophytes, *Pestalotiopsis* spp. has attracted much attention in recent years for their ability to produce a variety of bioactive secondary metabolites [7 – 9]. In a search for new bioactive natural products from this fungal genus, a subculture of *P. fici* (AS 3.9138 = W106-1), isolated from the branches of the tea plant *Camellia sinensis* (Theaceae) in the suburb of Hangzhou, P. R. China, was grown in different solid-substrate fermentation cultures. Previously, chemical studies of the resulting crude extracts led to the isolation of biologically active metabolites with interesting structural features [10 – 16], including five new meroterpenoids, pestalofones D – H (**4** – **8**; *cf. Scheme*), and their putative biosynthetic precursors, iso-A82775C (**9**) and isosulochrin (**10**; *cf. Scheme*) [15][16]. Since the HPLC finger-print of the crude extract revealed the presence of other minor analogs that could not be identified due to sample limitations, the fungus was refermented on a larger scale using the solid fermentation culture, in which the pestalofones D – H were isolated. The resulting AcOEt extract was separated exhaustively, affording additional three new meroterpenoids, which we named pestalofones I – K (**1** – **3**). Details of the isolation, structure elucidation, cytotoxicity, and plausible biogenesis of these compounds are reported herein.



1 (Racemate)



2 R = β-Me
3 R = α-Me

Results and Discussion. – *Structure Elucidation*. Pestalofone I (**1**) was assigned the molecular formula $C_{33}H_{34}O_{10}$ (17 degrees of unsaturation) on the basis of HR-ESI-MS (*m*/*z* 591.2228 ([*M* + H]$^+$); Δ – 0.3 mmu). The ^{1}H- and ^{13}C-NMR spectra (*Table 1*) showed resonances for four exchangeable H-atoms (δ(H) 12.4, 12.0, 9.00, and 8.12, resp.), six Me groups including two MeO, three CH_2, one O-bearing sp^3 quaternary C-atom, 20 aromatic/olefinic C-atoms (six of which were of the CH type), one ester C=O C-atom (δ(C) 166.1), and two ketone C=O C-atoms (δ(C) 198.9 and 203.8, resp.). These data accounted for all the ^{1}H- and ^{13}C-NMR resonances and required the compound to be tetracyclic. Comparison of the ^{1}H- and ^{13}C-NMR spectra

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of **1** with those of the known metabolite pestalofone G (**4**) [16] revealed that the presence of nearly identical structural features. Analysis of the HMBCs (*Fig. 1*) established the same partial structures (rings *C* and *D*) as found in **4** (*cf. Scheme*). The ^{1}H,^{1}H-COSY data showed one isolated spin system of C(4)–C(5). HMBCs from Me(1) and Me(2) to C(3) and C(4) enabled these two Me groups attached to C(3) of the C(3)=C(4) bond. HMBC Cross-peaks from CH_2(5) to C(6), C(7), and C(16), from H–C(7) to C(5), C(8), C(9), and C(16), from H–C(9) to C(7), C(8), C(11), and C(16), and from HO–C(16) to C(6), C(10), and C(16) established the tetrasubstituted benzene ring *A* with the CH_2(5) C-atom, two OH groups and the ketone C(11)=O C-atom attached to C(6), C(8), C(16), and C(10), respectively. Further HMBCs from Me(15) to C(12), C(13), and C(14) indicated that the sp^3 quaternary C-atom C(13) is connected to C(12), C(14), and C(15). Other correlations from CH_2(12) to C(11), and from CH_2(14) to C(4′), C(5′), and C(6′) led to the connection of the CH_2(12) C-atom to the ketone C(11)=O C-atom, and C(14) to C(5′), respectively. Considering the ^{13}C-NMR chemical shifts of C(13) (δ(C) 89.0) and C(6′) (δ(C) 159.4), as well as the tetracyclic nature of **1**, these two C-atoms have to be connected to the remaining O-atom to form a dihydrofuran moiety (ring *C*), thereby completing the gross structure of **1** as shown. The optical rotation of **1** was measured to be zero and no *Cotton* effect (CE) was observed in its ECD spectra, suggesting that **1** was a racemic mixture. However, a subsequent HPLC separation of **1** using a chiral column was unsuccessful. To the best of our knowledge, the fungal natural products isopestacin and fimetarone A were also reported to be racemic mixtures [17][18].

The elemental composition of pestalofone J (**2**) was established as $C_{33}H_{36}O_{11}$ (16 degrees of unsaturation) by HR-ESI-MS (*m*/*z* 609.2329 ([*M* + H]$^+$); Δ + 0.1 mmu), which is 18 mass units less than pestalofone G (**4**) [16]. Interpretation of the ^{1}H- and ^{13}C-NMR data (*Table 2*)

Table 1. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp., (D_6)acetone) *of* **1**. Arbitrary atom numbering indicated in the formulae; δ in ppm, *J* in Hz.

Position	δ(H)	δ(C)	HMBC
1	1.73 (*s*)	17.2	2, 3, 4
2	1.76 (*s*)	25.2	1, 3, 4
3		132.8	
4	5.34 (*t*, *J* = 6.5)	122.0	1, 2
5	3.32 (*d*, *J* = 6.5)	27.6	3, 4, 6, 7, 16
6		131.6	
7	7.03 (*s*)	124.9	5, 8, 9, 16
8		148.9	
9	7.03 (*s*)	112.4	7, 8, 11, 16
10		119.4	
11		203.8	
12	3.20 (*s*)	47.0	11
13		89.0	
14	2.88, 3.17 (2*d*, *J* = 15)	39.0	4′, 5′, 6′, 12, 13, 15
15	1.36 (*s*)	25.9	12, 13, 14
16		154.2	
1′		107.0	
2′		162.5	
3′	6.27 (*s*)	109.0	1′, 2′, 5′, 8′
4′		143.2	
5′		117.0	
6′		159.4	
7′		198.9	
8′	2.19 (*s*)	19.0	3′, 4′, 5′
1″		130.9	
2″		124.8	
3″		155.5	
4″	6.71 (*s*)	105.6	2″, 3″, 5″, 6″
5″		160.7	
6″	7.01 (*s*)	105.6	4″
7″		166.1	
8″	3.68 (*s*)	51.7	7″
9″	3.87 (*s*)	55.2	5″
HO–C(8)	8.12 (br. *s*)		
HO–C(16)	12.0 (*s*)		6, 10, 16
HO–C(2′)	12.4 (*s*)		1′, 2′, 3′
HO–C(3″)	9.00 (br. *s*)		

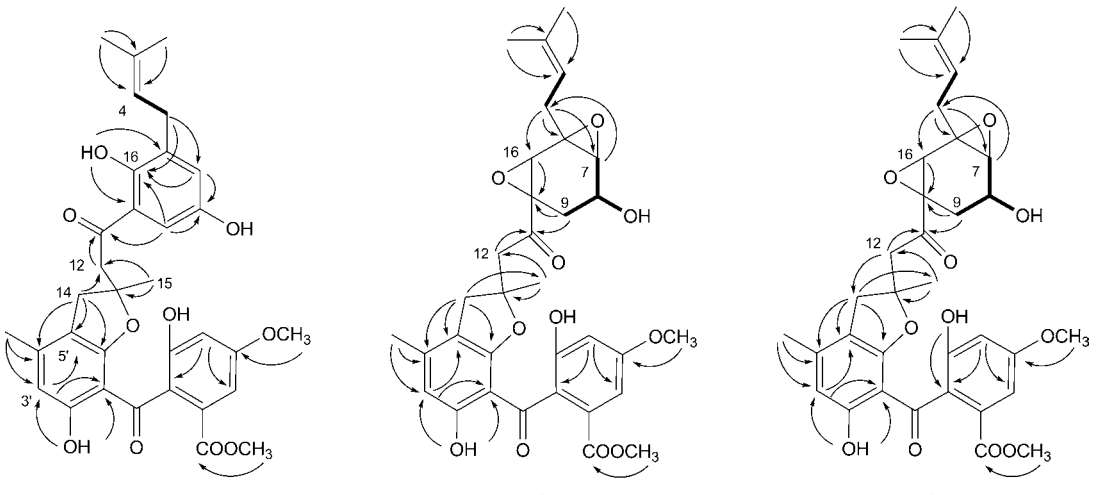

Fig. 1. *^{1}H,^{1}H-COSY* (—) *and selected HMBC* (H → C) *correlations of* **1**–**3**

Table 2. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp., (D_6)acetone) *of* **2** *and* **3**. Arbitrary atom numbering indicated in the formulae; δ in ppm, J in Hz.

Position	**2**			**3**	
	δ(H)	δ(C)	HMBC	δ(H)	δ(C)
1	1.63 (*s*)	18.1	2, 3, 4	1.68 (*s*)	18.1
2	1.71 (*s*)	25.9	1, 3, 4	1.75 (*s*)	25.9
3		136.3			136.3
4	5.22 (*t*, *J* = 7.5)	118.1		5.23 (*t*, *J* = 7.5)	117.7
5	2.42 (*dd*, *J* = 15, 7.5), 2.66 (*dd*, *J* = 15, 7.5)	33.8	3, 4, 6, 7, 16	2.42 (*dd*, *J* = 15, 7.5), 2.66 (*dd*, *J* = 15, 7.5)	33.9
6		57.9			57.9
7	3.08 (*s*)	60.8	5, 6, 8, 9	3.08 (*s*)	60.9
8	4.09 (*ddd*, *J* = 10, 6.0, 4.0)	67.6		4.09 (*ddd*, *J* = 10, 6.0, 4.0)	67.6
9	1.40 (*dd*, *J* = 14, 10), 2.46 (*dd*, *J* = 14, 6.0)	28.0	7, 8, 10, 11	1.40 (*dd*, *J* = 14, 10), 2.46 (*dd*, *J* = 14, 6.0)	27.9
10		61.7			61.6
11		205.4			205.2
12	2.45, 2.56 (2*d*, *J* = 18)	45.0	11, 13	2.35, 2.66 (2*d*, *J* = 18)	44.5
13		89.1			89.1
14	2.71, 2.81 (2*d*, *J* = 15)	39.2	4′, 5′, 6′, 12, 13, 15	2.71, 2.98 (2*d*, *J* = 15)	39.2
15	1.13 (*s*)	26.3	12, 13, 14	1.13 (*s*)	26.3
16	3.40 (br. *s*)	57.2	10	3.31 (br. *s*)	56.9
1′		107.5			107.5
2′		163.1			163.1
3′	6.20 (*s*)	109.5	1′, 5′, 8′	6.21 (*s*)	109.5
4′		143.8			143.8
5′		118.2			118.2
6′		159.9			159.9
7′		199.3			199.3
8′	2.11 (*s*)	19.6	3′, 4′, 5′	2.12 (*s*)	19.6
1″		130.9			130.9
2″		125.5			125.5
3″		156.1			156.1
4″	6.70 (*d*, *J* = 2.0)	106.4	2″, 5″, 6″	6.71 (*d*, *J* = 2.0)	106.4
5″		161.4			161.4
6″	7.04 (*d*, *J* = 2.0)	106.6	2″, 4″, 7″	7.03 (*d*, *J* = 2.0)	106.6
7″		166.6			166.6
8″	3.67 (*s*)	52.3	7″	3.68 (*s*)	52.3
9″	3.86 (*s*)	55.9	5″	3.87 (*s*)	55.9
HO–C(8)	4.15 (*d*, *J* = 4.0)		8	4.15 (*d*, *J* = 4.0)	
HO–C(2′)	12.4 (*s*)		1′, 2′, 3′	12.4 (*s*)	
HO–C(3″)	8.76 (*s*)			8.76 (*s*)	

revealed the presence of three exchangeable H-atoms (δ(H) 12.4, 8.76, and 4.15, resp.), six Me groups including two MeO, four CH_2, three O–CH, three O-bearing sp^3 quaternary C-atoms, 14 aromatic/olefinic C-atoms (four connected with one H-atom), one ester C=O C-atom (δ(C) 166.6), and two ketone C=O C-atoms (δ(C) 199.3 and 205.4, resp.). Comparison of the ^{1}H- and ^{13}C-NMR data with those of **4** revealed their structural similarity, except that the resonances for HO–C(10) (δ(H) 4.39) and HO–C(16) (δ(H) 3.65) had disappeared, and the ^{13}C-NMR chemical resonances for C(10) (δ(C) 61.7 in **2** *vs.* 80.4 in **4**) and C(16) (δ(C) 57.2 in **2** *vs.* 72.0 in **4**) were shifted upfield. The above-mentioned structural variation was confirmed by HMBCs (*Fig. 1*) from CH_2(5) to C(16) and from CH_2(9) to C(10). Therefore, **2** was established as a dehydration product of **4**. The relative configuration of **2** was deduced to be the same as that of **4** by comparison of its ^{1}H,^{1}H coupling constants and NOESY data (*Fig. 2*) with those of **4** [16]. The large *trans*-diaxial-type coupling constant of 10 Hz observed between H–C(8) and H_a–C(9) indicated that H–C(8) and H_a–C(9) are in a *pseudo*-axial orientation, and the vanishing coupling constant between H–C(7) and H–C(8) indicated H–C(7) in a *pseudo*-equatorial orientation with respect to the corresponding

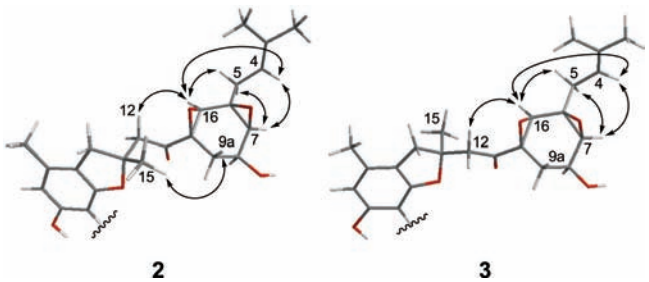

Fig. 2. *Key NOESY* (H ↔ H) *correlations of* **2** *and* **3**

Table 3. *Cytotoxic Activities of Compounds* **1** – **3**

Compound	IC_{50} [μM]			
	HeLa	T24	A549	MCF-7
1	> 50	> 50	> 50	> 50
2	44.3 ± 2.08	39.3 ± 2.72	35.2 ± 1.25	38.3 ± 1.17
3	65.5 ± 23.08	45.7 ± 4.61	58.9 ± 5.46	29.2 ± 2.03
Cisplatin	7.4 ± 0.90	3.9 ± 0.19	8.4 ± 0.29	6.37 ± 0.74

cyclohexanol ring. NOESY Correlations of H–C(7) with H–C(4) and CH_2(5), and of H–C(16) with H–C(4), CH_2(5) and H_a–C(12) indicated that these H-atoms are all on the same face of the ring system. Other correlations of H_a–C(9) with Me(15) revealed their proximity in space (*Fig. 2*), thereby completing the relative configuration of **2**. The absolute configuration of **2** was assumed to be same as that of **4**, considering their close structural relationship.

Pestalofone K (**3**) was assigned the same molecular formula $C_{33}H_{36}O_{11}$ (seven degrees of unsaturation) as **2** by HR-ESI-MS (*m*/*z* 609.2338 ($[M+H]^+$); Δ − 0.8 mmu). Interpretation of the ^{1}H- and ^{13}C-NMR data (*Table 2*) revealed the same constitution as that of **2**, which was supported by relevant ^{1}H,^{1}H-COSY and HMBC data, suggesting that **3** is a diastereoisomer of **2**. The epoxycyclohexanol moiety in **3** was also assigned the same relative configuration as that in **2**, **4**, and **5** by comparison of the ^{1}H,^{1}H coupling constants and NOESY data for relevant H-atoms (*Fig. 2*) [16], suggesting that **3** differs from **2** by having a different configuration at the stereogenic center C(13). The relative configuration at C(13) in **3** was deduced to be opposite to that in **2** on the basis of biosynthetic considerations, which was partially supported by the lack of a NOESY correlations of H_a–C(9) with Me(15). The absolute configuration of **3** was also deduced as shown by analogy to **2**.

Biological Studies. Compounds **1** – **3** were tested for cytotoxic activities against a panel of four human tumor cell lines, HeLa, T24, A549, and MCF-7 (*Table 3*). Compound **2** showed weak cytotoxic activities against all the cell lines tested, with the IC_{50} values of 44.3, 39.3, 35.3, and 38.3 μM, respectively (the positive control cisplatin showed the IC_{50} values of 7.4, 3.89, 8.4, and 6.4 μM, resp.), while compound **1** did not show any detectable cytotoxic activities at 50 μM.

Conclusions. – Pestalofone I (**1**) is a new meroterpenoid with 2-(7-benzoyl-2,3-dihydrobenzofuran-2-yl)-1-phenylethan-1-one skeleton. Compound **1** is structurally related to pestalofone G (**4**) [16], but differs by having a 2,5-dihydroxy-3-(3-methylbut-2-en-1-yl)phenyl unit at C(11) instead of a 2,3,5-trihydroxy-1-(3-methylbut-2-en-1-yl)-7-oxabicyclo[4.1.0]hept-3-yl subunit. Pestalofones J (**2**) and K (**3**) are new meroterpenoids possessing a 2-(7-benzoyl-2,3-dihydrobenzofuran-2-yl)-1-(3,8-dioxatricyclo [5.1.0.0^{2,4}]oct-4-yl)ethan-1-one scaffold. Compounds **2** and **3** are structurally related to pestalofones G (**4**) and H (**5**) [16], but differ by having the 3,8-dioxatricyclo[5.1.0.0^{2,4}]octane instead of the 7-oxabicyclo[4.1.0]heptane unit. Biogenetically, **1** – **3** could be derived from the known precursors iso-A82775C (**9**) and isosulochrin (**10**) [15][16] *via* a series of reactions, as illustrated in the *Scheme*. Natural products derived from **9** and **10** are rare. Precedents include pestalofones D – H (**4** – **8**) from *P. fici* in our previous study [15][16]. Compounds **1** – **3** are the dehydration products of **4** and **5**. The discovery of these new secondary metabolites further expanded the structural diversity of the bioactive products produced by the plant endophytic fungus *P. fici*.

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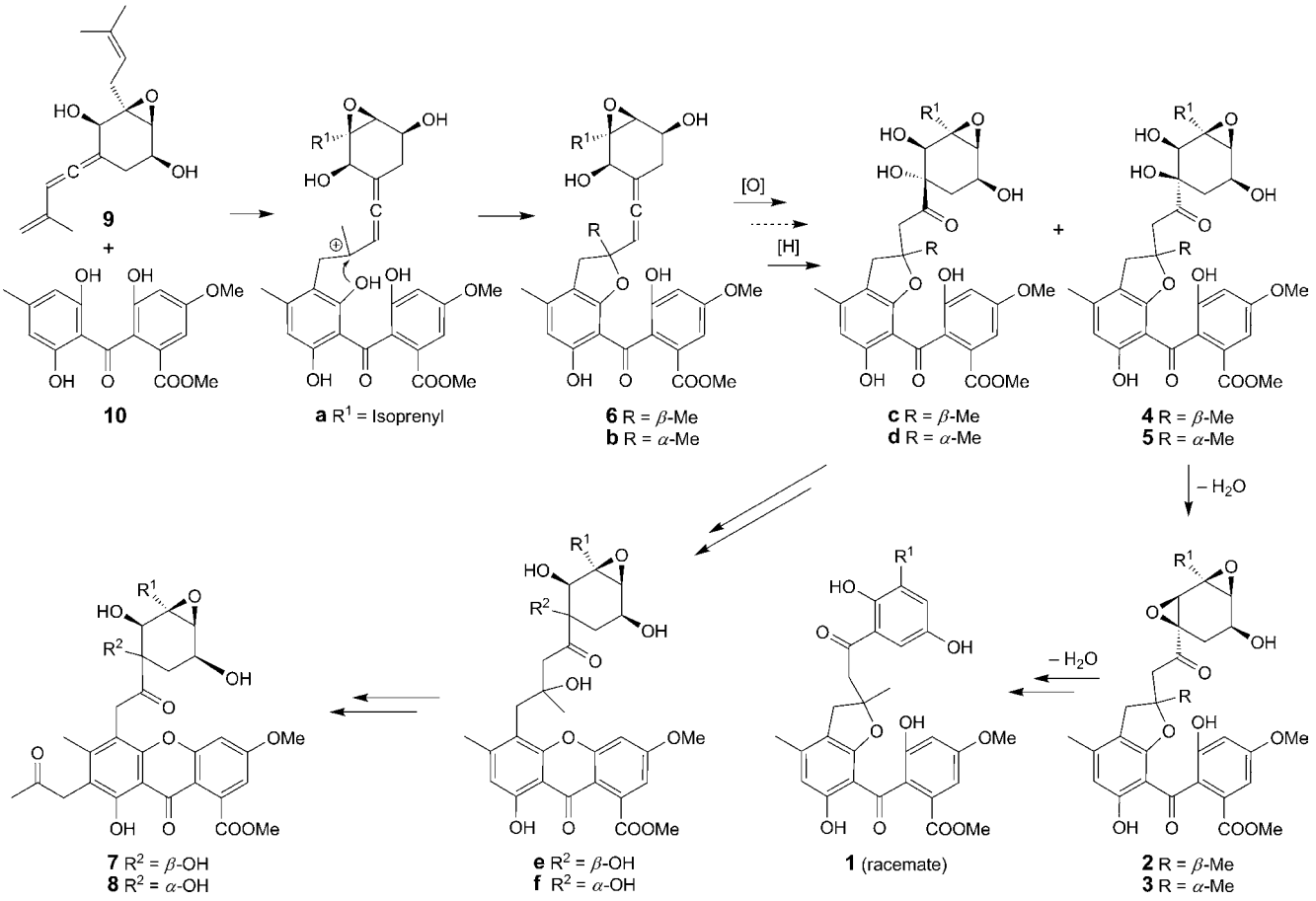
Experimental Part

General. Thin layer chromatography (TLC): silica gel GF_{254} (SiO_2). Column chromatography (CC): SiO_2 (200 – 300 mesh); *Sephadex LH-20* (*GE Healthcare Bio-sciences AB*). Semi-prep. reversed-phase (RP) HPLC: *Agilent 1200* liquid chromatography with a *Zorbax SB-C$_{18}$* column (5 μm; 9.4 × 250 mm); flow rate 2 ml/min. Optical rotations: *PerkinElmer 241* polarimeter spectropolarimeter. UV Spectra: *Shimadzu Biospec-1601* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet Magna-IR 750* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Varian Mercury-500* spectrometer, in (D_6)acetone; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Agilent Accurate-Mass-Q-TOF LC/MS 6520* spectrometer; in *m*/*z*.

Fungal Material. The culture of *P. fici* was isolated from the branches of *Camellia sinensis* (Theaceae) in a suburb of Hangzhou, P. R. China, in April 2005. The isolate was identified as *P. fici* by one of the authors (*L. G.*) based on sequence (GenBank Accession number DQ812914) analysis of the ITS region of the ribosomal DNA and assigned the accession number AS 3.9138 (= W106-1) in the 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° for 10 d. Agar plugs were cut into small pieces (*ca.* 0.5 × 0.5 × 0.5 cm^3) under aseptic conditions, 15 pieces were used to inoculate three *Erlenmeyer* flasks (250 ml), each containing 50 ml of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract); the final pH of the media was adjusted to 6.5 and sterilized by autoclave. Three flasks of the inoculated media were incubated at 25° on a rotary shaker at 170 rpm for 5 d to prepare the seed culture. Spore inoculum was prepared by suspension in sterile, dist. H_2O to give a final spore/cell suspension of 1 × 10^6/ml. Fermentation was carried out in 36 *Fernbach* flasks (500 ml), each containing 80 g of rice. Dist. H_2O (120 ml) was added to each flask, and the contents were soaked overnight before autoclaving at 15 psi for 30 min. After cooling to r.t., each flask was inoculated with 5.0 ml of the spore inoculum and incubated at 25° for 40 d.

Scheme. *Proposed Biosynthetic Pathway for* **1**–**8**



Extraction and Isolation. The fermented material was extracted with AcOEt (4 × 3.0 l) for 7 d, and the org. solvent was evaporated to dryness under vacuum to afford the crude extract (33 g), which was fractionated by vacuum liquid chromatography (VLC; SiO_2; petroleum ether (PE)/AcOEt gradient). The fraction (600 mg) eluted with 24% AcOEt was separated by CC (*Sephadex LH-20*; CH_2Cl_2/MeOH 1:1). The resulting subfractions were combined and further purified by semi-prep. RP HPLC (60–70% MeCN in H_2O for 30 min) to afford **1** (1.8 mg; t_R 24.7 min). The fraction eluted with 33% AcOEt (560 mg) was separated by CC (*Sephadex LH-20*; CH_2Cl_2/MeOH 1:1). The subfractions were combined and further purified by RP HPLC (48–50% MeCN in H_2O for 40 min) to afford **2** (1.7 mg; t_R 31.5 min), and **3** (3.1 mg; t_R 33.5 min).

Pestalofone I (= *Methyl 2-[(2-{2-[2,5-Dihydroxy-3-(3-methylbut-2-en-1-yl)phenyl]-2-oxoethyl}-2,3-dihydro-6-hydroxy-2,4-dimethyl-1-benzofuran-7-yl)carbonyl]-3-hydroxy-5-methoxybenzoate*; **1**). Yellow powder. UV (MeOH): 214 (3.99), 268 (3.72). IR (neat): 3392 (br.), 2928, 1717, 1639, 1444, 1364, 1240, 1181, 1046. ^{1}H-, ^{13}C-NMR, and HMBC: see *Table 1*. HR-ESI-MS: 591.2228 ($[M+H]^+$, $C_{33}H_{35}O_{10}^+$; calc. 591.2225).

Pestalofone J (= *Methyl 2,3-Dihydro-3-hydroxy-2-{[(2*R*)-6-hydroxy-2-{2-[(1*S*,2*S*,4*R*,6*S*,7*S*)-6-hydroxy-1-(3-methylbut-2-en-1-yl)-3,8-dioxatricyclo[5.1.0.0^{2,4}]oct-4-yl]-2-oxoethyl}-2,4-dimethyl-1-benzofuran-7-yl]carbonyl}-5-methoxybenzoate*; **2**). Yellow powder. $[\alpha]_D^{25} = -24.1$ ($c = 0.17$, MeOH). UV (MeOH): 220 (4.01), 278 (3.84). IR (neat): 3400 (br.), 2926, 1717, 1639, 1605, 1446, 1241, 1025. ^{1}H- and ^{13}C-NMR: see *Table 2*. Key HMBC and ^{1}H,^{1}H-COSY correlations ((D$_6$)acetone, 500 MHz): see *Fig. 1*. NOESY Correlations ((D$_6$)acetone, 500 MHz): see *Fig. 2*. HR-ESI-MS: 609.2329 ($[M+H]^+$, $C_{33}H_{37}O_{11}^+$; calc. 609.2330).

Pestalofone K (= *Methyl 2,3-Dihydro-3-hydroxy-2-{[(2*S*)-6-hydroxy-2-{2-[(1*S*,2*S*,4*R*,6*S*,7*S*)-6-hydroxy-1-(3-methylbut-2-en-1-yl)-3,8-dioxatricyclo[5.1.0.0^{2,4}]oct-4-yl]-2-oxoethyl}-2,4-dimethyl-1-benzofuran-7-yl]carbonyl}-5-methoxybenzoate*; **3**). Yellow powder. $[\alpha]_D^{25} = +6.5$ ($c = 0.31$, MeOH). UV (MeOH): 224 (3.85), 278 (3.80). IR (neat): 3400 (br.), 2928, 1716, 1639, 1609, 1445, 1238, 1025. ^{1}H- and ^{13}C-NMR: see *Table 2*. Key HMBC and ^{1}H,^{1}H-COSY correlations ((D$_6$)acetone, 500 MHz): see *Fig. 1*. NOESY Correlations ((D$_6$)acetone, 500 MHz): see *Fig. 2*. HR-ESI-MS: 609.2338 ($[M+H]^+$, $C_{33}H_{37}O_{11}^+$; calc. 609.2330).

MTS Assay [19]. In a 96-well plate, each well was plated with (2–5) × 10^3 cells (depending on the cell multiplication rate). After cell attachment overnight, the medium was removed, and each well was treated with 100 µl of medium containing 0.1% DMSO, or appropriate concentrations of the test compounds and the positive control cisplatin (100 mM as stock soln. of a compound in DMSO and serial dilutions; the test compounds showed good solubility in DMSO and did not precipitate when added to the cells). The plate was incubated for 48 h at 37° in a humidified, 5% CO_2 atmosphere. Proliferation assessed by adding 20 µl of MTS (*Promega*) to each well in the dark, followed by a 90-min incubation at 37°. The assay plate was read at 490 nm using a microplate reader. The assay was run in triplicate.

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