Bioorganic & Medicinal Chemistry 17 (2009) 7362–7367

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Cytosporones, coumarins, and an alkaloid from the endophytic fungus *Pestalotiopsis* sp. isolated from the Chinese mangrove plant *Rhizophora mucronata*

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ARTICLE INFO

Article history:
Received 21 April 2009
Revised 11 August 2009
Accepted 13 August 2009
Available online 20 August 2009

Keywords:
Cytosporones
Coumarins
Alkaloid
Pestalotiopsis sp.
Spectroscopic data analysis

ABSTRACT

Chemical examination of the endophytic fungus *Pestalotiopsis* sp., isolated from the leaves of the Chinese mangrove *Rhizophora mucronata*, yielded 11 new compounds including cytosporones J–N (**1–3, 5–6**), five new coumarins pestalasins A–E (**8–12**), and a new alkaloid named pestalotiopsoid A (**14**), along with the known compounds cytosporone C (**4**), dothiorelone B (**7**), and 3-hydroxymethyl-6,8-dimethoxycoumarin (**13**). The structures of the new compounds were unambiguously elucidated on the basis of extensive spectroscopic data analysis.

1. Introduction

Fungi of the genus *Pestalotiopsis* are known as endophytes of tropical higher plants.[1–3] Since discovery of the anticancer agent taxol from an endophytic fungal strain of the genus *Pestalotiopsis*,[4,5] interest in bioactive compounds from this fungal genus has increased considerably. Previous chemical investigations of *Pestalotiopsis* spp. led to the discovery of various bioactive natural products such as polyketides and terpenoids.[6–14] In our ongoing search for new bioactive metabolites from fungal endophytes,[15,16] we recently described the isolation and identification of several new chromone derivatives from an undescribed fungal strain of the genus *Pestalotiopsis* sp. isolated from leaves of the Chinese mangrove plant *Rhizophora mucronata*.[17] Continued investigation of the minor constituents obtained after fermentation of the fungus on solid rice medium now afforded a further series of new as well as a number of known compounds. The structures of all these were unequivocally determined by one- and two-dimensional NMR spectroscopy as well as by mass spectrometry and by comparison with the literature data.

2. Result and discussion

The mycelia and culture medium of the endophytic fungus *Pestalotiopsis* sp. were extracted with ethyl acetate. This extract was concentrated and then repeatedly chromatographed over silica gel and Sephadex LH-20 followed by preparative HPLC to yield 11 new compounds, including cytosporones J–N (**1–3, 5–6**), pestalasins A–E (**8–12**), and pestalotiopsoid A (**14**), together with the known derivatives cytosporone C (**4**),[18] dothiorelone B (**7**),[19] and 3-hydroxymethyl-6,8-dimethoxycoumarin(**13**).[20]

Cytosporone J (**1**), a colorless amorphous solid, has the molecular formula $C_{16}H_{22}O_5$, established by HR-ESI-MS (*m/z* 295.1538, calcd for $[M+H]^+$ 295.1540), implying six degrees of unsaturation. The ^1H NMR data of **1** (Table 1) and its ^1H–^1H COSY spectrum (Fig. 1) exhibited an oxygenated methine signal at δ_H 3.68 (m, CH-15), two *meta*-coupled aromatic protons at δ_H 6.23 (br s, H-4)

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doi:10.1016/j.bmc.2009.08.031

Table 1
^{1}H NMR (500 MHz) data (*J* in Hz) of cytosporones J–N

Position	**1**[a]	**2**[b]	**3**[b]	**5**[a]	**6**[a]
2	3.79, d, 19.6 3.45, d, 19.6	3.79, d, 18.9 3.45, d, 19.25	3.77, d, 19.2 3.44, d, 19.55	3.67, s	3.67, s
4	6.23, br s	6.24, s	6.22, s	6.30, d, 2.3	6.31, d, 1.8
5-OH	8.74 br s				
6	6.34, d, 1.5	6.35, d, 1.55	6.33, d, 1.85	6.37, d, 2.3	6.37, d, 1.8
7-OH	8.37 br s				
9	5.56, dd, 8.7, 5.1	5.56, dd, 9.0, 4.9	5.53, dd, 9.0, 5.3		
10	1.86, m 1.79, m	1.92, m 1.80, m	1.92, m 1.80, m	2.92, t, 7.3	2.89, t, 6.2
11	1.55, m 1.34–1.47, m	1.55, m 1.28–1.40, m	1.25–1.47, m	1.62, m	1.62, m
12	1.34–1.47, m	1.28–1.40, m	1.25–1.47, m	1.27–1.47, m	1.29–1.32, m
13	1.34–1.47, m	1.28–1.40, m	1.25–1.47, m	1.27–1.47, m	1.29–1.32, m
14	1.34–1.47, m	1.28–1.40, m	1.25–1.47, m	1.27–1.47, m	1.29–1.32, m
15	3.68, br s	4.82, m	1.25–1.47, m	3.68, m	1.29–1.32, m
16	1.08, d, 6.3	1.16, d, 6.3	3.98, t, 6.6	1.09, d, 6.3	0.88, t, 5.8
17				3.61, s	3.62, s
18		2.20, s	2.20, s		

[a] In acetone-d_6.
[b] In methanol-d_4.

and 6.34 (d, J = 1.5 Hz, H-6), two geminal protons at δ_H 3.45 (d, J = 19.6, H-2a) and 3.79 (d, J = 19.6 Hz, H-2b), an oxygenated methine at δ_H 5.56 (dd, J = 8.7, 5.1 Hz, H-9), and the signals of a seven-membered alkyl chain from CH_2-10 to CH_3-16. These ^{1}H NMR data were very similar to those reported for cytosporone C (**4**) previously isolated from the endophytic fungus *Cytospora* sp. CR200,[18] suggesting that both compounds shared the same basic skeleton, except for the presence of an additional oxymethine proton (δ_H 3.68, m, H-15) in the side chain of **1**. The COSY correlation of the terminal methyl protons (δ_H 1.08, d, J = 6.3, H_3-16) to the neighboring oxymethine proton revealed the extra hydroxyl group is bound to C-15. Thus, **1** was 15-hydroxycytosporone C.

Cytosporone K (**2**) was found to have the molecular formula $C_{18}H_{24}O_6$, established by HR-ESI-MS (*m/z* 337.1646, calcd for $[M+H]^+$ 337.1651), which is 43 amu larger than that of **1** and suggested the presence of an acetyl group from the observation of the fragment at *m/z* 277 in the ESI-MS spectrum, derived from loss of CH_3COOH. The ^{1}H NMR data of **2** (Table 1) were similar to those of **1**, except for the significant downfield shift of H-15 (δ_H 4.82, m) and presence of a methyl signal at δ_H 2.2 (s, H_3-18) indicative of acetylation of the hydroxyl substituent at C-15. Hence **2** was 15-acetoxycytosporone C.

Cytosporone L (**3**) had the same molecular formula as **2** and the ESI-MS fragment at *m/z* 277 again suggested the loss of CH_3COOH, indicating that **3** is an isomer of **2**. The ^{1}H NMR data (Table 1) were similar to those of the known cytosporone C (**4**),[18] except for the methyl signal of an acetyl group at δ_H 2.2 (3H, s) and the signal of an oxymethylene group (δ_H 3.98, t, J = 6.6, H_2-16) that replaces the terminal methyl group of **4**. This clearly established the position of the acetoxy group at C-16 rather than at C-15 as observed in **2**. Hence **3** was 16-acetoxycytosporone C.

Cytosporone M (**5**) has the molecular formula $C_{17}H_{24}O_6$, established by HR-ESI-MS (*m/z* 325.1654, calcd for $[M+H]^+$ 325.1646), indicating five degrees of unsaturation. The ^{1}H NMR data of **5** (Table 1) and its ^{1}H–^{1}H COSY (Fig. 1) displayed a methoxy singlet at δ_H 3.61 (s, H_3-17), a methylene at δ_H 3.67 (s, H_2-2), an oxygenated methine at δ_H 3.68 (m, H-15), two *meta*-coupled aromatic protons at δ_H 6.30 (d, J = 2.3 Hz, H-4); δ_H 6.37 (d, J = 2.3 Hz, H-6), and the resonances for a seven-membered alkyl chain (CH_2-10 to CH_3-16). The ^{1}H NMR data of **5** were very similar to those of dothiorelone B (**7**), previously isolated from the endophytic fungus *Dothiorella* sp. HTF3.[19] The only difference between these was replacement of the ethyl group of **7** by a methyl group in **5**. Hence **5** was 1-methoxy-1-deethoxy-dothiorelone B.

HR-ESI-MS data of cytosporone N (**6**) differed from that of **5** by 16 amu, suggesting the lack of a hydroxyl group. The ^{1}H NMR data (Table 1) and COSY spectrum of **6** indicated the compound featured the same core structure as **5** and possessed an unsubstituted heptyl side chain as in cytosporone A.[18] This was confirmed by the triplet signal of the terminal methyl group at δ_H 0.88 (t, J = 5.8 Hz, H_3-16). Hence cytosporone N was 15-dehydroxy cytosporone M.

Clearly the acyclic derivatives **5**–**7** are closely related to the cyclized cytosporones **1**–**4**, and their probable biogenetic relationship is depicted in Figure 5.

Pestalasin A (**8**), a colorless amorphous solid, has the molecular formula $C_{19}H_{24}O_5$, established by HR-ESI-MS (*m/z* 265.1074, calcd for $[M+H]^+$ 265.1071), suggesting seven degrees of unsaturation. The ^{1}H and ^{13}C NMR data of **8** (Table 2) indicated that six of the seven units of unsaturation could be due to an aromatic ring, a double bond, and one carbonyl group. Thus, the remaining unit of unsaturation was attributed to a ring formation. The UV absorption maxima at 208, 228, and 289 nm suggested **8** is a coumarin derivative. The ^{1}H and ^{13}C NMR data of **8** and its ^{1}H–^{1}H COSY spectrum exhibited two methoxy groups (δ_H 3.82, s, δ_C 55.8, q, 6-OCH_3; δ_H 3.92, s, δ_C 56.3, q, 8-OCH_3), two *meta*-coupled aromatic protons [δ_H 6.43 (d, J = 2.6 Hz), δ_C 99.8, d, CH-5; δ_H 6.64 (d, J = 2.6 Hz), δ_C 102.6, d, CH-7], an olefinic singlet (δ_H 7.52, s, δ_C 141.3, d, CH-4), and a 2-hydroxypropyl group (CH_2-1′ to CH_3-3′). Comparison of the NMR data of **8** with those of 6,8-dimethoxy-3-(2′-oxo-propyl)-coumarin,[21] previously isolated from the endophytic fungus *Periconia atropurpurea* associated with *Xylopia aromatica*, revealed that both compounds differed only with regard to the nature of the side chain at C-3, where the 2′-oxo-propyl group of the latter was replaced by the 2′-hydroxypropyl group of **8**. This was confirmed from COSY correlations and HMBC correlations (Fig. 2) from H-1′ (δ_H 2.55, 2.71) to C-2 (δ_C 162.2), C-3 (δ_C 127.3), and C-4 (δ_C 141.3). The HMBC and ROESY correlations (Figs. 2 and 3) supported the assignments of the methoxy groups 6-OCH_3 (δ_H 3.82, s) at C-6 (δ_C 156.4), and 8-OCH_3 (δ_H 3.92, s) at C-8 (δ_C 148.0) Accordingly, the structure of pestalasin A (**8**) was 6,8-dimethoxy-3-(2′-hydroxy-propyl)-coumarin.

Pestalasin B (**9**) had the molecular formula $C_{14}H_{16}O_6$, established by HR-ESI-MS (*m/z* 281.1029, calcd for $[M+H]^+$ 281.1025),

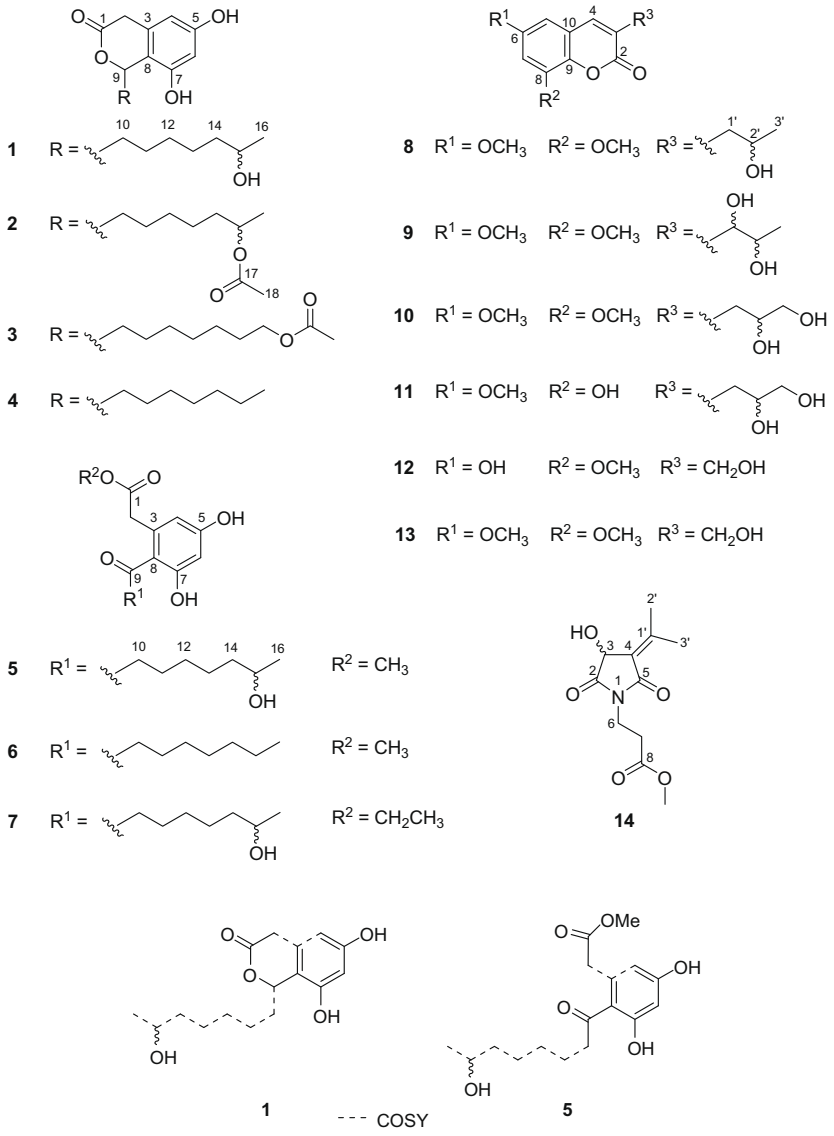

Figure 1. Key ^{1}H–^{1}H COSY correlations of **1** and **5**.

which is 16 amu larger than that of **8**. The ^{1}H NMR data of **9** (Table 2) closely resembled those of **8**, suggesting that both compounds have the same basic molecular framework. The proton signals of the side chain methylene group [δ_H 2.55 (dd, J = 8.0 Hz, J = 14.0 Hz), δ_H 2.71 (dd, J = 3.3 Hz, J = 14.0 Hz), 1′-CH_2] in **8** were absent in **9** and one additional hydroxymethine signal (δ_H 4.59, d, J = 7.0 Hz) indicated the presence of a second hydroxyl group at C-1′ in **9**. The COSY correlations from H-2′ to H-1′ and H-3′ revealed the presence of a 1′,2′-dihydroxypropyl group. Fragments at *m/z* 263 and 245 in the positive ESI-MS spectrum (Fig. 4) originated from the sequential loss of two molecules of water, further supporting the side chain assignment. Hence pestalasin B was 6,8-dimethoxy-3-(1′,2′-dihydroxypropyl)-coumarin.

Pestalasin C (**10**) has the same molecular formula as **9**, suggesting **10** is an isomer of **9**. The ^{1}H and ^{13}C NMR data of **10** (Table 2) were similar to those of **8** except for replacement of the 3′-CH_3 group in **8** by a CH_2OH group [δ_H 3.38, (dd, J = 6.2 Hz, J = 11.4 Hz), δ_H 3.48 (dd, J = 4.0 Hz, J = 11.4 Hz); δ_C 65.8, t, 3′-CH_2]. The ^{1}H–^{1}H COSY and HMBC correlations further supported the presence a 2′,3′-dihydroxypropyl group at C-3. Hence pestalasin C (**10**) was 6,8-dimethoxy-3-(2′,3′-dihydroxy-propyl)-coumarin.

The ^{1}H NMR data of pestalasin D (**11**) (Table 2) were similar to those of **10**, except for the absence of one of the two methoxy groups present in **10**. HR-ESI-MS data of **11** (*m/z* 267.0863 $[M+H]^+$) established the molecular formula of $C_{14}H_{16}O_6$, indicating replacement of one of the two methoxy groups of **10** by a hydroxyl group. Comparison with the chemical shifts of the methoxy substituents of **8**, unequivocally assigned by HMBC and ROESY spectra, allowed the assignment of the methoxy group (δ_H 3.80, s) of **11** to C-6. Hence pestalasin D was 8-hydroxy-6-methoxy-3-(2′,3′-dihydroxy-propyl)-coumarin.

The molecular formula $C_{11}H_{10}O_5$ of pestalasin E (**12**) was established by HR-ESI-MS (*m/z* 223.0606, calcd for $[M+H]^+$ 223.0601). Its ^{1}H NMR data (Tables 2) were closely related to those of the known analogue 3-hydroxymethyl-6,8-dimethoxycoumarin (**13**) previously isolated from *Talaromyces flavus*,[21] except for the presence of only one methoxy group at δ_H 3.91 (s), which was assigned by comparison to C-8 rather than to C-6. Hence pestalasin E was 6-hydroxy-8-methoxy-3-propyl-coumarin.

Pestalotiopsoid A (**14**) was obtained as a colorless oil. Its molecular formula $C_{11}H_{15}NO_5$ was established by HR-ESI-MS *m/z* 242.1020 $[M+H]^+$ (calcd for $C_{11}H_{16}NO_5$, 242.1023), indicating five

Table 2
^{1}H, ^{13}C NMR (500 MHz) data (*J* in Hz) of pestalasins A–C

Position	**8**[b]		**9**[a]	**10**[c]		**11**[a]	**12**[a]	**13**[b]	
	δ_H	δ_C		δ_H	δ_C			δ_H	δ_C
2		162.2, s			162.2, s				162.2, s
3		127.3, s			126.2, s				127.3, s
4	7.52, s	141.3, d	7.94, s	7.53, s	142.0, d	7.72, s	7.79, s	7.67, s	141.3, d
5	6.43, d, 2.6	99.8, d	6.69, d, 2.6	6.37, d, 2.6	99.8, d	6.64, d, 2.85	6.64, d, 2.5	6.48, d, 2.5	99.8, d
6		156.4, s			156.4, s				156.4, s
7	6.64, d, 2.6	102.6, d	6.83, d, 2.6	6.54, d, 2.6	102.2, d	6.68, d, 2.85	6.75, d, 2.5	6.65, d, 2.5	102.6, d
8		148.0, s			148.0, s				148.0, s
9		119.9, s			119.8, s				119.9, s
10		138.1, s			138.0, s				138.1, s
1′	2.55, dd, 8.0, 14.0 2.71, dd, 3.3, 14.0	40.9, t	4.59, d, 7.0	2.48, dd, 8.2, 14.2 2.67, dd, 4.2, 14.2	34.2, t	2.51, dd, 9.0,13.55 2.80, m	4.49, s	4.62, s	40.9, t
2′	4.15, m	66.7, d	4.1, m	3.83, m	70.0, d	3.91, m			66.7, d
3′	1.27, d, 6.2	23.6, q	1.25, d, 6.3	3.38, dd, 6.2, 11.4 3.48, dd, 4.0, 11.4	65.8, t	3.52, d, 6.3 3.50, d, 5.05			23.6, q
6-OCH_3	3.82, s	55.8, q	3.88, s	3.81, s	55.8, q	3.80, s		3.84, s	55.8, q
8-OCH_3	3.92, s	56.3, q	3.96, s	3.89, s	56.3, q		3.91, s	3.94, s	56.3, q

[a] In acetone-d_6.
[b] In $CDCl_3$.
[c] In $CDCl_3$ + 10% methanol-d_4.

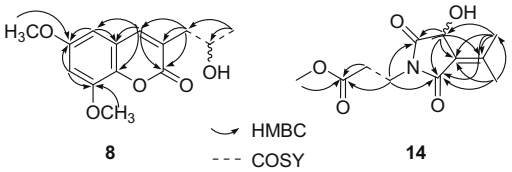

Figure 2. Key HMBC and ^{1}H–^{1}H COSY correlations of **8** and **14**.

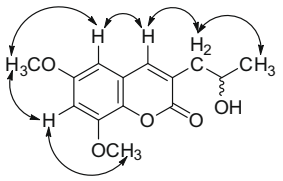

Figure 3. Key ROESY correlations of **8**.

degrees of unsaturation. The ^{1}H and ^{13}C NMR data of **14** revealed that four of the five units of unsaturation were attributed by three carbonyls and one double bond, thus, the remaining unit of unsaturation comes from a ring. The ^{1}H and ^{13}C NMR data of **14** in association with its ^{1}H–^{1}H COSY and HMQC spectra indicated a methoxy group (δ_H 3.65, s, δ_C 51.2, q, 8-OCH_3), two methyl groups [δ_H 2.1, s, δ_C 23.4, q, 3′-CH_3; δ_H 2.39, (d, *J* = 1.25 Hz), δ_C 21.0, q, 2′-CH_3] which showed homoallylic coupling to an oxymethine (δ_H 4.85, s, δ_C 67.5, d, H-3), and a 1,2-disubstituted ethyl group [δ_H 2.65 (t, *J* = 7.25 Hz), δ_C 31.7, 7-CH_2; δ_H 3.85 (t, *J* = 7.25 Hz), δ_C 33.9, 6-CH_2]. The HMBC correlations (Fig. 2) from H-3 to both amidocarbonyl carbons at δ_C 174.6 (s, C-2) and δ_C 167.4 (s, C-5) together with C-4 (δ_C 121.1) and C-1′ (δ_C 157.3), combined with that from 2′-CH_3 and 3′-CH_3 to C-5, C-4 and C-1′, established the 3-hydroxy-2,5-pyrrolidinedione nucleus and the connection of two methyl groups to C-1′. Moreover, a methyl propanoate group was assigned through the HMBC correlation from the methoxy protons and the protons of 1,2-disubstituted ethyl group to C-8 (δ_C 170.7). This moiety was linked to the nitrogen atom of the 2,5-pyrrolidinedione unit based on the observed HMBC correlations from 6-H_2 to both amidocarbonyl carbons C-2 and C-5. Thus, pestalotiopsoid A was methyl-3-(3-hydroxy-2,5-dioxo-4-(propan-2-ylidene)pyrrolidin-1-yl)propanoate. Due to the low amounts of the compound isolated, it was not possible to assign the stereochemistry of the chiral derivatives.

Previously other fungal cytosporone derivatives that bear structural similarities to compounds **5–7** isolated in our study had been described to be cytotoxic against several cancer cell lines in vitro.[19] This prompted us to investigate the cytotoxicity of compounds **1–13** against three cancer cell lines including L5178 Y, Hela and PC12 cells. However, in contrast to the previous report[19] none of the compounds investigated showed any significant activity when tested at an initial concentration of 10 μg/mL. Due to the minute amounts of compounds isolated from *Pestalotiopsis* sp. no further bioactivity studies could be performed.

3. Experimental section

3.1. General experimental procedures

Optical rotation was recorded using a Perkin–Elmer Model 341 LC polarimeter. UV spectral data were obtained from online UV spectra measured by photodiode array detection (Gynkotek, Ger-

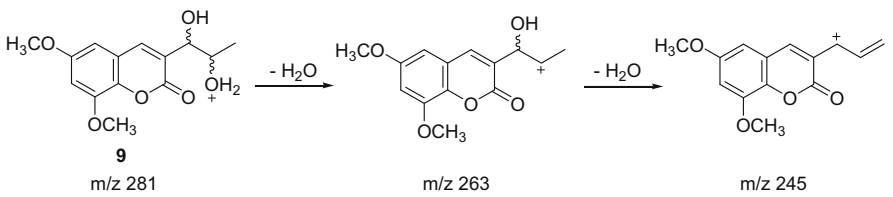

Figure 4. ESI-MS fragments of compound **9**.

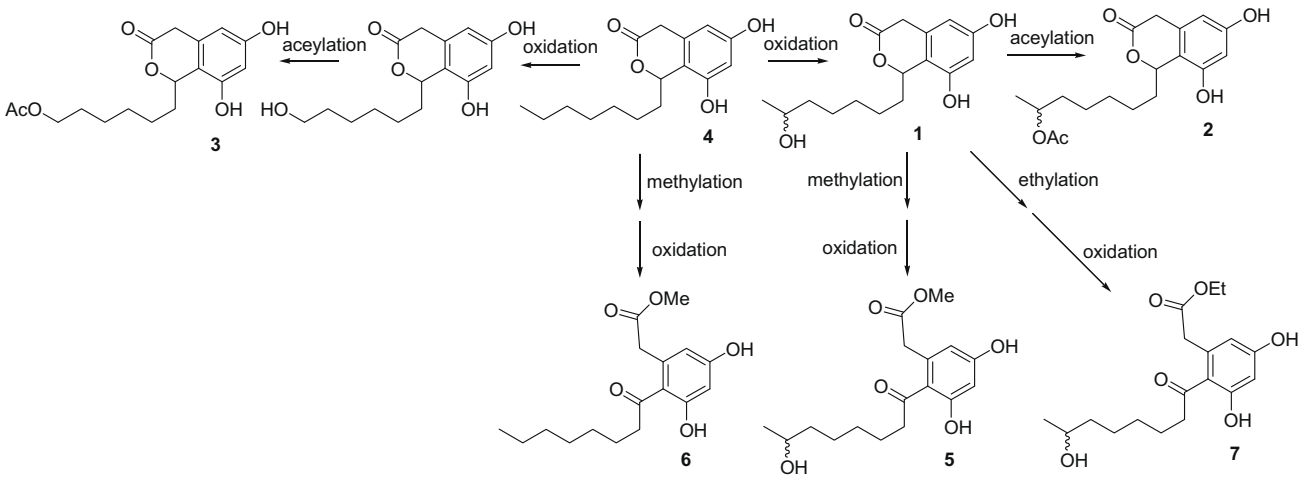

Figure 5. Proposed biogenetic relationship of cytosporones **1**–**7**.

many). ^{1}H and ^{13}C NMR (chemical shifts in ppm) spectra were recorded on Bruker ARX 600 or DRX 500 NMR spectrometers in acetone-d_6, methanol-d_4 or $CDCl_3$. ESI-MS spectra were recorded on a Finnigan MAT TSQ 7000 mass spectrometer. High-resolution ESI-MS were recorded on a Micromass Q-Tof-2 mass spectrometer using peak matching.

3.2. Isolation and cultivation of the fungus

Pestalotiopsis sp. was isolated from fresh, healthy leaf material of *R. mucronata* (Rhizophoraceae) collected in October 2005 in Dong Zhai Gang-Mangrove Garden on Hainan Island, China. The fungus (strain no. JCM2A4) was isolated under sterile conditions from the inner tissue of the leaf following an isolation protocol described previously[15] and identified using a molecular biological protocol by DNA amplification and sequencing of the ITS region.[22] The fungus was grown on solid rice medium at room temperature under static conditions for 39 days. The sequence data have been submitted to and deposited at GenBank (accession no. FJ465172). A voucher strain (Code No. 2) was deposited at the Institut für Pharmazeutische Biologie und Biotechnologie, Heinrich-Heine-Universität, Duesseldorf.

3.3. Extraction and isolation

The mycelia and solid rice medium were extracted with EtOAc. The extract was evaporated under reduced pressure to yield 3.0 g residue. This residue was subjected to vacuum liquid chromatography (VLC) on a silica gel column employing a step gradient of dichloromethane–methanol. Each fraction containing 50 mL was dried and examined by TLC on premade silica gel plates (Merck, Germany) using a dichloromethane–methanol based solvent system. Moreover, each fraction obtained was analyzed by HPLC using a reversed-phase column and employing a linear gradient of methanol and water (adjusted to pH 2.0 by addition of phosphoric acid). Promising fractions were subjected to further chromatographic separation using Sephadex LH-20 with methanol as solvent. Final purification was achieved by semi-preparative reversed-phase HPLC to yield **1** (1.09 mg), **2** (0.76 mg), **3** (0.66 mg), **4** (3.48 mg), **5** (0.30 mg), **6** (0.90 mg), **7** (0.82 mg), **8** (2.45 mg), **9** (0.33 mg), **10** (1.38 mg), **11** (0.30 mg), **12** (0.79 mg), **13** (1.92 mg), **14** (0.86 mg).

3.4. Cytotoxicity assay

Cytotoxicity activity was evaluated against L5178Y, Hela and PC12 cells by the MTT method.[23] The cell lines were grown in RPMI-1640 culture medium with Na-carbonate (pH 7.2) supplemented with 10% FCS (fetal calf serum) under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. An aliquot (180 μL) of these cell suspensions at a density of 1500 cell mL^{-1} was pipetted into 96-well microtiter plates. Subsequently, 180 μL of the test compounds (in DMSO) at different concentrations was added to each well and incubated for 72 h at the above conditions in a CO_2-incubator. MTT solution (20 μL of 5 mg/mL in RPMI-1640 medium) was added to each well and further incubated for 3 h. After addition of 100 μL DMSO and incubation for 1 h, the cells were lysed to liberate the formed formazan crystals. Absorbance was then determined on Multiscan plate reader at 595 nm. As negative controls, media with 0.1% EGMME/DMSO were included in all experiments.

3.4.1. Cytosporone J (1)

Colorless amorphous residue (MeOH); $[\alpha]_D^{20}$ +11 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 203, 281 nm; ^{1}H NMR data, see Table 1; HR-ESI-MS *m/z* 295.1538 $[M+H]^+$ (calcd for $C_{16}H_{23}O_5$, 295.1540).

3.4.2. Cytosporone K (2)

Colorless amorphous residue (MeOH); $[\alpha]_D^{20}$ +7 (*c* 0.02, MeOH); UV (MeOH) λ_{max} 203, 280 nm; ^{1}H NMR data, see Table 1; HR-ESI-MS *m/z* 337.1646 $[M+H]^+$ (calcd for $C_{18}H_{25}O_6$, 337.1651).

3.4.3. Cytosporone L (3)

Colorless amorphous residue (MeOH); $[\alpha]_D^{20}$ −8 (*c* 0.02, MeOH); UV (MeOH) λ_{max} 203, 281 nm; ^{1}H NMR data, see Table 1; HR-ESI-MS *m/z* 337.1652 $[M+H]^+$ (calcd for $C_{18}H_{25}O_6$, 337.1646).

3.4.4. Cytosporone C (4)

Colorless amorphous residue (MeOH); $[\alpha]_D^{20}$ +11 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 208, 282 nm; ^{1}H NMR(500 MHz, acetone-d_6) δ 8.73 (1H, br s, 5-OH), 8.36 (1H, br s, 7-OH), 6.34 (1H, d, J = 0.6 Hz, H-6), 6.23 (1H, d, J = 0.6 Hz, H-4), 5.55 (1H, dd, J = 8.8 Hz, J = 5.0 Hz, H-9), 3.79 (1H, d, J = 19.2 Hz, H-2), 3.45 (1H, d, J = 19.2 Hz, H-2), 1.86 (1H, m, H-10), 1.79 (1H, m, H-10), 1.55 (1H, m, H-11), 1.42 (1H, m, H-11), 1.27–1.40 (8H, m, H12–15), 0.87 (3H, t, J = 6.9 Hz, H-16); ^{13}C NMR (125 MHz, acetone-d_6) δ 170.5 (C-1), 159.0 (C-5), 155.0 (C-7), 133.0 (C-3), 114.0 (C-8), 106.3 (CH-6), 101.0 (CH-4),

78.3 (CH-9), 36.4 (CH_2-10), 35.4 (CH_2-2), 32.5 (CH_2-14), 29.9 (CH_2-13), 29.5 (CH_2-12), 26.3 (CH_2-11), 23.0 (CH_2-15), 14.2 (CH_3-16); ESI-MS $[M+H]^+$ *m/z* 279 ($C_{16}H_{23}O_4$).

3.4.5. Cytosporone M (5)

Colorless amorphous residue (MeOH); $[\alpha]_D^{20}$ −10 (*c* 0.02, MeOH); UV (MeOH) λ_{max} 220, 272, 299 nm; ^{1}H NMR data, see Table 1; HR-ESI-MS *m/z* 325.1654 $[M+H]^+$ (calcd for $C_{17}H_{25}O_6$, 325.1646).

3.4.6. Cytosporone N (6)

Colorless amorphous residue (MeOH); UV (MeOH) λ_{max} 202, 220, 270 nm; ^{1}H NMR data, see Table 1; HR-ESI-MS *m/z* 309.1694 $[M+H]^+$ (calcd for $C_{17}H_{25}O_5$, 309.1697).

3.4.7. Dothiorelone B (7)

Colorless amorphous residue (MeOH); $[\alpha]_D^{20}$ −6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 220, 269, 299 nm; ^{1}H NMR(500 MHz, acetone-d_6) δ 6.36 (1H, d, *J* = 2.2 Hz, H-6), 6.30 (1H, d, *J* = 2.2 Hz, H-4), 4.07 (1H, q, *J* = 7.3 Hz, H-17), 3.68 (1H,m, H-15), 3.67 (2H, s, H-2), 2.92 (2H, t, *J* = 7.3, H-10), 1.62 (2H, m, H-11), 1.34-1.47 (6H, m, H12–14), 1.20 (3H, t, *J* = 7.3 Hz, H-18), 1.08 (3H, t, *J* = 6.3 Hz, H-16); HR-ESI-MS *m/z* 339.1809 $[M+H]^+$ (calcd for $C_{18}H_{27}O_6$, 339.1802).

3.4.8. Pestalasin A (8)

Yellow amorphous residue (MeOH); $[\alpha]_D^{20}$ +22.7 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 208, 228, 289 nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HR-ESI-MS *m/z* 265.1074 $[M+H]^+$ (calcd for $C_{14}H_{17}O_5$, 265.1071).

3.4.9. Pestalasin B (9)

Yellow amorphous residue (MeOH); $[\alpha]_D^{20}$ +12 (*c* 0.02, MeOH); UV (MeOH) λ_{max} 208, 228, 292 nm; ^{1}H NMR data, see Table 1; HR-ESI-MS *m/z* 281.1029 $[M+H]^+$ (calcd for $C_{14}H_{17}O_6$, 281.1025).

3.4.10. Pestalasin C (10)

Yellow amorphous residue (MeOH); $[\alpha]_D^{20}$ −13 (*c* 0.2, MeOH); UV (MeOH) λ_{max} 209, 229, 290 nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HR-ESI-MS *m/z* 281.1026 $[M+H]^+$ (calcd for $C_{14}H_{17}O_6$, 281.1020).

3.4.11. Pestalasin D (11)

Yellow amorphous residue (MeOH); $[\alpha]_D^{20}$ +6 (*c* 0.02, MeOH); UV (MeOH) λ_{max} 208, 223, 293 nm; ^{1}H NMR data, see Table 1; HR-ESI-MS *m/z* 267.0863 $[M+H]^+$ (calcd for $C_{13}H_{15}O_6$, 267.0869).

3.4.12. Pestalasin E (12)

Yellow amorphous residue (MeOH); UV (MeOH) λ_{max} 207, 227, 289 nm; ^{1}H NMR data, see Table 1; HR-ESI-MS *m/z* 223.0606 $[M+H]^+$ (calcd for $C_{11}H_{11}O_5$, 223.0601).

3.4.13. 3-Hydroxymethyl-6,8-dimethoxycoumarin (13)

Yellow amorphous residue (MeOH); UV (MeOH) λ_{max} 208, 228, 289 nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2. ESI-MS $[M+HCOOH]^+$ *m/z* 282 ($C_{12}H_{12}O_5$ + HCOOH).

3.4.14. Pestalotiopsoid A (14)

A colorless oil (MeOH); $[\alpha]_D^{20}$ −13 (*c* 0.02, MeOH); UV (MeOH) λ_{max} 207, 240, 505 nm; ^{1}H NMR(500 MHz, $CDCl_3$) δ 4.85 (1H, s, H-3), 3.85 (2H, t, *J* = 7.3 Hz, H-6), 2.65 (2H, t, *J* = 7.3 Hz, H-7), 3.65 (3H, s, OMe-8), 2.10 (3H, s, Me-2′), 2.39 (3H, d, *J* = 1.3, H-2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.4 (C-2), 67.5 (CH-3), 121.1 (C-4), 174.6 (C-5), 33.9 (CH_2-6), 31.5 (CH_2-7), 179.7 (C-8), 51.2 (8-OCH_3), 157.3 (C-1′), 23.4 (CH_3-2′), 21.0 (CH_3-3′); ESI-MS $[M+H]^+$ *m/z* 279 ($C_{16}H_{23}O_4$). HR-ESI-MS *m/z* 242.1020 $[M+H]^+$ (calcd for $C_{11}H_{16}NO_5$, 242.1023).

Acknowledgments

Financial support by grants of the BMBF and MOST (2006AA09Z446) to Peter Proksch and Wenhan Lin is gratefully acknowledged. Jing Xu wishes to thank the China Scholarship Council of the Ministry of Education of China for a scholarship.

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