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Petiolins A–C, phloroglucinol derivatives from *Hypericum pseudopetiolatum* var. *kiusianum*

Naonobu Tanaka,[a] Takaaki Kubota,[a] Haruaki Ishiyama,[a] Atsushi Araki,[a] Yoshiki Kashiwada,[b] Yoshihisa Takaishi,[b] Yuzuru Mikami[c] and Jun'ichi Kobayashi[a,*]

[a]*Graduate School of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan*
[b]*Graduate School of Pharmaceutical Sciences, University of Tokushima, Tokushima 770-8505, Japan*
[c]*Medical Mycology Research Center, Chiba University, Chiba 260-0856, Japan*

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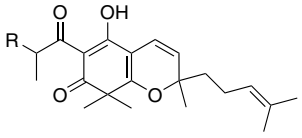
Abstract—Two new phloroglucinol derivatives possessing chromane skeleton, petiolins A (**1**) and B (**2**), and a new phloroglucinol derivative containing a dihydrofuran ring, petiolin C (**3**), were isolated from aerial parts of *Hypericum pseudopetiolatum* var. *kiusianum*. The gross structures of **1**–**3** were elucidated by spectroscopic data, and the relative stereochemistry of **3** was elucidated by NOESY data. Petiolins A–C (**1**–**3**) showed modest cytotoxicity, while petiolin C (**3**) exhibited antifungal activity.

1. Introduction

The genus *Hypericum* (family Clusiaceae) are known to be a traditional medicine for the treatment of burns, bruises, swelling, inflammation, and anxiety as well as bacterial and viral infections.[1–4] In our continuing search for bioactive compounds from *Hypericum* spp.,[5–10] three new phloroglucinol derivatives, petiolins A–C (**1**–**3**), were isolated from aerial parts of *Hypericum pseudopetiolatum* var. *kiusianum*. In this paper, we describe the isolation and structure elucidation of **1**–**3**.

2. Results and discussion

The aerial parts of *H. pseudopetiolatum* var. *kiusianum* were extracted with MeOH, and the extracts were partitioned between *n*-hexane and H_2O. *n*-Hexane-soluble portions were subjected to a silica gel column chromatography (*n*-hexane/EtOAc) and then a Sephadex LH-20 column (EtOH) to afford a mixture of phloroglucinol derivatives, which was purified by C_{18} column (MeOH/H_2O) and C_{18} HPLC (MeOH/H_2O) to yield petiolins A (**1**, 0.0017%), B (**2**, 0.0022%), and C (**3**, 0.006%).



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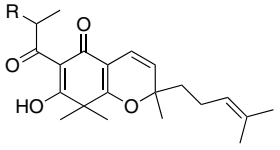
2a: R = Et



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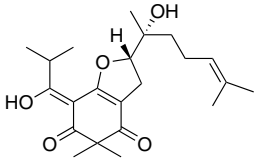

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The molecular formula, $C_{22}H_{30}O_4$, of petiolin A (**1**) was established by HRFABMS [*m*/*z* 359.2235 $(M+H)^+$, Δ +1.3 mmu]. IR absorptions implied the presence of hydroxyl (3441 cm^{-1}) and carbonyl (1739 and 1660 cm^{-1}) functionalities. Two lower-field signals of hydrogen-bonded hydroxyl protons at δ_H 19.15 and 18.72 in a ratio of 4:1 in the ^1H NMR spectrum were observed, indicating the presence of two enol tautomers **1a** and **1b** in the ratio of 4:1. ^{13}C NMR data (Table 1) of major tautomer **1a** revealed the presence of two ketone carbonyl carbons (δ_C 207.9 and 196.0), five sp^2 quaternary carbons (δ_C 185.8, 173.7, 132.2, 104.3, and 102.3), three sp^2 methines (δ_C

Keywords: *Hypericum pseudopetiolatum* var. *kiusianum*; Phloroglucinol derivatives; Petiolins A–C.
* Corresponding author. Tel.: +81 11 706 3239; fax: +81 11 706 4989; e-mail: jkobay@pharm.hokudai.ac.jp

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Table 1. ^{1}H and ^{13}C NMR data for major (**1a**) and minor (**1b**) tautomers of petiolin A (**1**) in $CDCl_3$

Position	1a		1b	
	^{13}C	^{1}H[a]	^{13}C	^{1}H[a]
1	25.7	1.68 (3H, s)	25.7	1.68 (3H, s)[b]
2	132.2	—	132.2	—
3	17.5	1.59 (3H, s)	17.5	1.59 (3H, s)[b]
4	123.2	5.09 (1H, t, J = 7.3)	123.2	5.09 (1H, m)[b]
5	22.6	2.06 (2H, m)	22.6	2.06 (2H, m)[b]
6	41.5	1.79 (1H, m), 1.64 (1H, m)	41.4	1.79 (1H, m)[b], 1.64 (1H, m)[b]
7	83.6	—	81.8	—
8	27.2	1.41 (3H, s)	26.7	1.37 (3H, s)
9	122.2	5.30 (1H, d, J = 9.8)	122.4	5.28 (1H, d, J = 9.7)
10	115.4	6.52 (1H, d, J = 9.8)	116.6	6.59 (1H, d, J = 9.7)
11	102.3	—	107.5	—
12	185.8	—	180.6	—
13	104.3	—	107.5	—
14	196.0	—	198.5	—
15	48.1	—	43.1	—
16	173.7	—	166.7	—
17	23.7	1.39 (3H, s)	24.4	1.50 (3H, s)
18	24.3	1.38 (3H, s)	23.8	1.49 (3H, s)
19	207.9	—	210.2	—
20	35.3	3.96 (1H, sept, J = 6.2)	36.0	4.24 (1H, sept, J = 7.0)
21	18.9	1.16 (3H, d, J = 6.2)	18.9	1.19 (3H, d, J = 7.0)
22	18.9	1.16 (3H, d, J = 6.2)	18.9	1.19 (3H, d, J = 7.0)
12 or 14-OH	—	19.15 (1H, s)	—	18.72 (1H, s)

[a] Coupling constants given (J, Hz) in parentheses.
[b] Overrapped with signals of major tautomer.

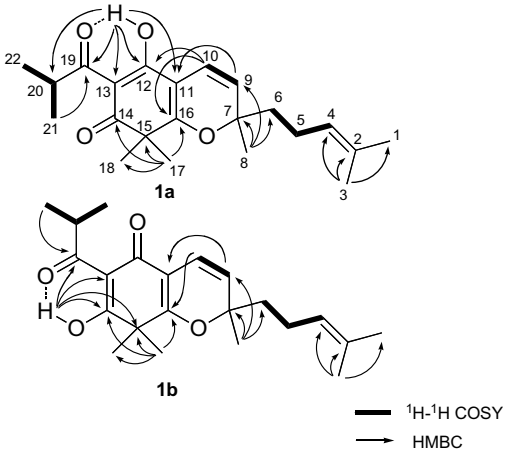


Figure 1. Selected 2D NMR correlations for major (**1a**) and minor (**1b**) tautomers of petiolin A (**1**). Hydrogen bonds are illustrated by broken lines.

123.2, 122.2, and 115.4), two sp^3 quaternary carbons (δ_C 83.6 and 48.1), one sp^3 methine (δ_C 35.3), two sp^3 methylenes (δ_C 41.5 and 22.6), and seven methyls (δ_C 27.2, 25.7, 24.3, 23.7, 18.9, 18.9, and 17.5). These signals were similar to those of major tautomer of hyperguinone B[11] except for chemical shifts of C-7 and C-15, indicating that **1a** had different substituents at C-7 and C-15 from those of hyperguinone B. ^{1}H–^{1}H COSY cross-peaks of H_2-5 to H-4, and H_2-6, and HMBC correlations of H_3-3 to C-1, C-2, and C-4, and H_3-8 to C-6, C-7, and C-9 (Fig. 1) indicated that **1a** had a 4-methyl-3-pentenyl group at C-7. Connectivities of C-15 to C-17 and C-18 were elucidated from HMBC correlations of H_3-17 to C-14, C-15, C-16, and C-18 (Fig. 1). Thus, the gross structure of major tautomer (**1a**) of **1** was assigned as shown in Figure 1. Similarly, the gross structure of minor tautomer **1b** was elucidated by a comparison of the ^{1}H and ^{13}C NMR data (Table 1) with those of minor tautomer of hyperguinone B,[11] in addition to the analysis of the ^{1}H–^{1}H COSY and HMBC spectra of **1b**.

Petiolin B (**2**) showed the pseudomolecular ion peak at m/z 373 $(M+H)^+$ in the FABMS, and the HRFABMS analysis revealed the molecular formula to be $C_{23}H_{32}O_4$ [m/z 373.2378 $(M+H)^+$, Δ −0.1 mmu], larger by 14 mass units as compared with petiolin A (**1**). Except for signals of an acyl side chain at C-13, ^{1}H and ^{13}C NMR data of petiolin B (**2**) were similar to those of **1** (Tables 1 and 2). ^{1}H NMR signals due to two tautomeric forms (**2a** and **2b**) in the ratio of 4:1 in $CDCl_3$ were observed. The side chain of **2** was assigned as 2-methylbutanoyl group by correlations of H-20 to H_3-21 and H_2-22, and H_2-22 to H_3-23 in the ^{1}H–^{1}H COSY spectrum, and H_3-21 to C-19 in the HMBC spectrum. The connectivity of C-13 to C-19 was deduced from HMBC correlations of OH-12 to C-19 and C-20 in **2a**, and OH-14 to C-19 in **2b**. Thus, the gross structures of enol tautomers of **2a** and **2b** were elucidated as shown in Figure 2.

The CD spectra for petiolins A (**1**) [λ_{ext} 302 ($\Delta\varepsilon$ −0.1), 277 (+0.1), and 223 nm (−0.3)] and B (**2**) [λ_{ext} 303 ($\Delta\varepsilon$ −0.1), 275 (+0.1), and 216 nm (−0.3)] were opposite to those of known related compounds, blandachromenes I and II,[12] whose absolute configurations at C-7 were assigned by the CD data. However, it was difficult to discuss the stereochemistry of C-7 in **1** and **2** on the basis of the CD data, since substitution patterns of the chromane skeleton in petiolins A (**1**) and B (**2**) were different from those of blandachromenes I and II.

Table 2. ^{1}H and ^{13}C NMR data for major (**2a**) and minor (**2b**) tautomers of petiolin B (**2**) in $CDCl_3$

Position	2a		2b	
	^{13}C	^{1}H[a]	^{13}C	^{1}H[a]
1	25.4	1.68 (3H, s)	25.4	1.68 (3H, s)[b]
2	132.2	—	132.2	—
3	17.5	1.59 (3H, s)	17.5	1.59 (3H, s)[b]
4	123.2	5.09 (1H, t, J = 7.3)	123.2	5.09 (1H, t, J = 7.3)[b]
5	22.6	2.07 (2H, m)	22.6	2.07 (2H, m)[b]
6	41.5	1.80 (1H, m), 1.64 (1H, m)	41.6	1.80 (1H, m), 1.64 (1H, m)[b]
7	83.6	—	81.8	—
8	27.2	1.41 (3H, s)	26.8	1.37 (3H, s)
9	122.2	5.30 (1H, d, J = 10.1)	122.2	5.28 (1H, d, J = 10.1)
10	115.4	6.52 (1H, d, J = 10.1)	116.6	6.59 (1H, d, J = 10.1)
11	102.4	—	107.2	—
12	185.9	—	180.6	—
13	105.0	—	107.2	—
14	196.2	—	198.7	—
15	48.1	—	43.2	—
16	173.6	—	166.7	—
17	23.7	1.39 (3H, s)	24.5	1.50 (3H, s)
18	24.3	1.38 (3H, s)	23.9	1.49 (3H, s)
19	209.9	—	209.9	—
20	41.6	3.83 (1H, qt, J = 6.7, 6.7)	42.3	4.13 (1H, qt, J = 6.9, 6.9)
21	16.5	1.14 (3H, d, J = 6.7)	16.5	1.17 (3H, d, J = 6.9)
22	26.6	1.76 (1H, m), 1.41 (1H, m)	26.6	1.76 (1H, m),[b] 1.41 (1H, m)[b]
23	11.7	0.94 (3H, br t, J = 6.7)	11.7	0.94 (3H, m)[b]
12 or 14-OH	—	19.19 (1H, br s)	—	18.79 (1H, s)

[a] Coupling constants given (J, Hz) in parentheses.
[b] Overrapped with the signals of major tautomer.

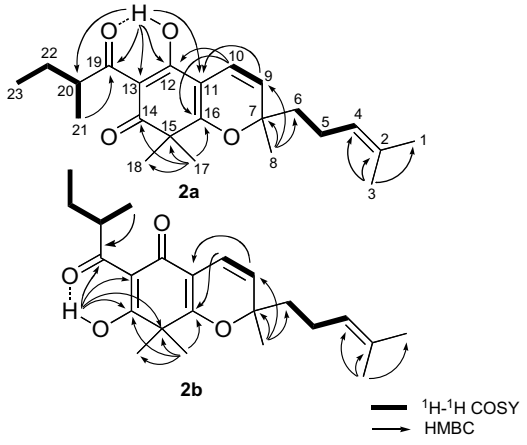


Figure 2. Selected 2D NMR correlations for major (**2a**) and minor (**2b**) tautomers of petiolin B (**2**). Hydrogen bonds are illustrated by broken lines.

The molecular formula, $C_{22}H_{32}O_5$, of petiolin C (**3**) was established by the HRFABMS [*m*/*z* 377.2312 $(M+H)^+$, Δ −1.6 mmu]. The IR spectrum implied the presence of hydroxyl (3413 cm^{-1}) and resonance-stabilized enol form of β-diketone (1595 cm^{-1})[13] functionalities. The gross structure of **3** was deduced from detailed analysis of ^{1}H and ^{13}C NMR data (Table 3) aided with 2D NMR experiments (^{1}H–^{1}H COSY, HMQC, and HMBC) as shown in Figure 3. The ^{13}C NMR(Table 3) spectrum disclosed the existence of two ketone carbonyl carbons (δ_C 205.9 and 193.0), five sp^2 quaternary carbons (δ_C 199.5, 168.9, 132.7, 106.5, and 99.5), one sp^2 methine (δ_C 123.8), two sp^3 quaternary carbons (δ_C 73.6 and 52.9), two sp^3 methines (δ_C 92.0 and 34.9), three sp^3 methylenes (δ_C 37.7, 26.3, and 22.0), and seven methyls (δ_C 25.8, 25.5, 24.2, 22.8, 20.1, 19.2, and 17.8). The ^{1}H–^{1}H COSY spectrum revealed connectivities of C-9 to C-10, and C-4 to C-6. HMBC correlations of H_3-8 to C-6, C-7 (δ_C 73.6), and C-9 indicated that an oxygenated sp^3 quaternary carbon (C-7) was attached to C-8, C-6, and C-9. Connectivities from C-10 to C-12 and C-16 through C-11 were deduced from HMBC correlations of H_2-10 to C-12, C-11, and C-16.

HMBC correlations of H_3-17 to C-14, C-15, C-16, and C-18 suggested connectivities from C-17 to C-14, C-16, and C-18 through C-15. The chemical shift of C-9 (δ_C 92.0) indicated the presence of an ether linkage between C-9 and C-12, which was supported by the HMBC correlation of H-9 to C-12. The chemical shift of an sp^2 quaternary carbon C-13 (δ_C 99.5) suggested that this carbon was attached to C-12, C-14, and C-19. Thus, the gross structure of petiolin C was elucidated to be **3**.

The relative stereochemistry of petiolin C (**3**) was deduced from NOESY data measured in acetone-d_6. NOESY correlations of H-9 (δ_H 4.89) to H-6 (δ_H 1.55) and H-8 (δ_H 1.28), H-5 (δ_H 2.15) to H-10α (δ_H 2.77), and H-6 to H-10β (δ_H 2.89) suggested that the relative stereochemistry of C-7 and C-9 in **3** was as shown in Figure 4. The proposed relative stereochemistry of **3** was supported by resemblance of ^{13}C chemical shifts for C-7 of **3** with those of the corresponding position in bonannione B,[14] whose relative stereochemistry was elucidated by combined approach of spectroscopic means and quantum mechanical method.[14]

Table 3. ^{1}H and ^{13}C NMR data for petiolin C (**3**) in $CDCl_3$

Position	^{13}C	^{1}H[a]
1	25.8	1.69 (3H, s)
2	132.7	—
3	17.8	1.63 (3H, s)
4	123.8	5.12 (1H, t, J = 7.0)
5	22.0	2.16 (1H, m), 2.09 (1H, m)
6	37.7	1.53 (2H, m)
7	73.6	—
8	22.8	1.30 (3H, s)
9	92.0	4.79 (1H, t, J = 9.6)
10	26.3	2.88 (2H, m)
11	106.5	—
12	168.9	—
13	99.5	—
14	205.9	—
15	52.9	—
16	193.0	—
17	25.5	1.39 (3H, s)
18	24.2	1.38 (3H, s)
19	199.5	—
20	34.9	3.66 (1H, sept, J = 6.7)
21	20.1	1.21 (3H, d, J = 6.7)
22	19.2	1.23 (3H, d, J = 6.7)
OH-14	—	18.24 (1H, br s)

[a] Coupling constants given (J, Hz) in parentheses.

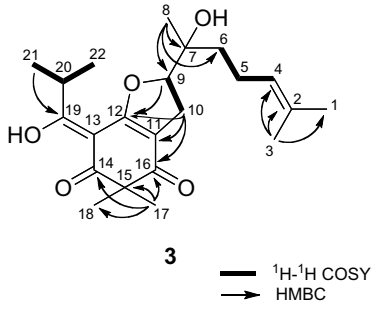


Figure 3. Selected 2D NMR correlations for petiolin C (**3**).

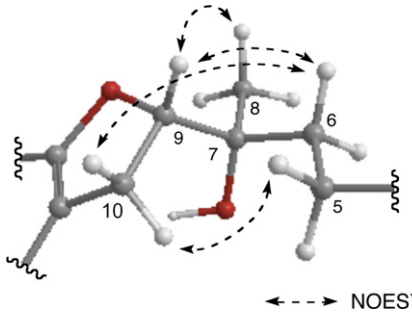


Figure 4. Selected NOESY correlations and relative stereochemistry for a partial structure (C-5–C-12) of petiolin C (**3**).

A plausible biogenetic path for petiolins A (**1**), B (**2**), and C (**3**) is proposed as shown in Scheme 1. It is reported that hyperforin, adhyperforin, and acylphloroglucinol derivatives from *H. perforatum* may be generated from amino acid precursors via a polyketide mechanism.[15–17] In the present plant, such an intermediate (**X**) proposed for the polyketide mechanism[16] seems to be similarily biosynthesized and followed by methylation, geranylation, epoxidation of a double bond, and intermolecular cyclization to generate petiolins A–C (**1**–**3**).

Petiolins A–C (**1**–**3**) exhibited cytotoxicity against murine lymphoma L1210 cells (IC_{50}, 2.5, >10, and 3.3 μg/mL, respectively) and human epidermoid carcinoma KB cells (IC_{50}, 4.8, 9.6, and 4.9 μg/mL, respectively) in vitro. Petiolin C (**3**) showed inhibitory activity against *Trichophyton mentagrophytes* (MIC, 33.3 μg/mL).

3. Experimental

3.1. General

Optical rotations were recorded on a JASCO P-1030 digital polarimeter. IR and UV spectra were recorded on JASCO FT/IR-230 and Shimadzu UV-1600PC spectrophotometers, respectively. NMR spectra were measured with a JEOL ECA 500 spectrometer. The 7.27 and 76.9 ppm resonances of residual $CDCl_3$ were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. FABMS spectra were recorded on a JEOL JMS-HX110 using glycerol as a matrix.

3.2. Plant material

Hypericum pseudopetiolatum var. *kiusianum* was collected in August 2005 in Kochi Prefecture, Japan, and identified by Dr. T. Akiyama (The Kochi Prefectural Makino Botanical Garden). Herbarium specimens were deposited in the botanical garden of the University of Tokushima (specimen number: UTP98013).

3.3. Extraction and isolation

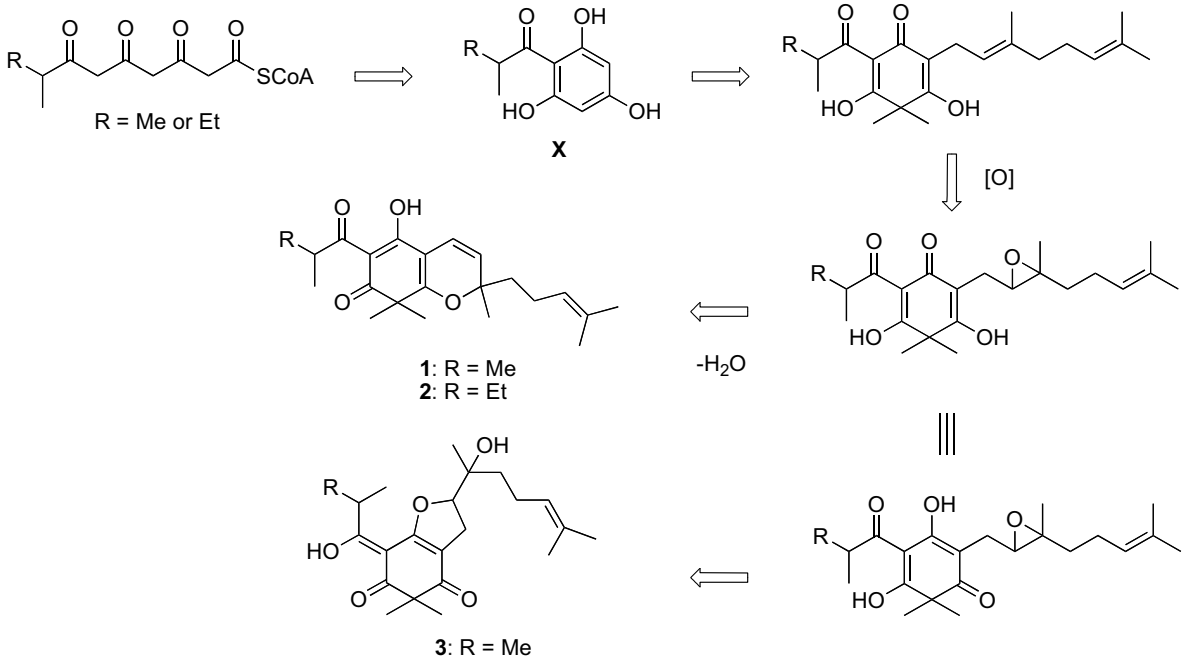
The aerial parts of *H. pseudopetiolatum* var. *kiusianum* (320 g) were extracted with MeOH (3× 3 L), and the extracts were partitioned between *n*-hexane (3× 300 mL) and H_2O (300 mL). The *n*-hexane-soluble portions were subjected to a silica gel column chromatography (*n*-hexane/EtOAc), a Sephadex LH-20 column (EtOH), a C_{18} column (MeOH/H_2O, 85: 15), and then C_{18} reversed-phase HPLC (Mightysil RP-18, Kanto Chemical Co. Ltd, 10 × 250 mm; flow rate 3.0 mL/min; UV detection at 254 nm; eluent MeOH/H_2O, 9:1) to afford petiolins A (**1**, 5.5 mg), B (**2**, 7.1 mg), and C (**3**, 19.2 mg).

3.4. Petiolin A (1)

Yellowish oil; $[\alpha]_D^{23}$ −0.7 (*c* 0.4 MeOH); UV (MeOH) λ_{max} 242 (ε 12,000), 269 (10,100), and 344 (2300) nm; CD (MeOH) $\Delta\varepsilon_{302}$ −0.1, $\Delta\varepsilon_{275}$ +0.1, and $\Delta\varepsilon_{223}$ −0.3; IR (KBr) ν_{max} 3441, 1739, and 1660 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); FABMS *m/z* 359 $(M+H)^+$; HRFABMS: *m/z* 359.2235 $(M+H)^+$ (Calcd for $C_{22}H_{31}O_4$, 359.2222).

3.5. Petiolin B (2)

Yellowish oil; $[\alpha]_D^{24}$ −1.6 (*c* 0.2 MeOH); UV (MeOH) λ_{max} 242 (ε 5000), 270 (6100), and 345 (2100) nm; CD



Scheme 1. Plausible biogenetic path of petiolins A–C (**1**–**3**).

(MeOH) $\Delta\varepsilon_{303}$ −0.1, $\Delta\varepsilon_{275}$ +0.1, and $\Delta\varepsilon_{216}$ −0.3; IR (KBr) ν_{max} 3508, 1739, and 1659 cm^{-1}; ^1H and ^{13}C NMR data (Table 2); FABMS *m/z* 373 $(M+H)^+$; HRFABMS: *m/z* 373.2378 $(M+H)^+$ (Calcd for $C_{23}H_{33}O_4$, 373.2379).

3.6. Petiolin C (3)

Yellowish oil; $[\alpha]_D^{23}$ 0 (*c* 1.3 MeOH); UV (MeOH) λ_{max} 244 (ε 7400), 320 (9600), and 342 (6400) nm; IR (KBr) ν_{max} 3413 and 1595 cm^{-1}; ^1H and ^{13}C NMR data (Table 3); FABMS *m/z* 377 $(M+H)^+$; HRFABMS: *m/z* 377.2312 $(M+H)^+$ (Calcd for $C_{22}H_{33}O_5$, 377.2328).

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