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Periconianone A, a New 6/6/6 Carbocyclic Sesquiterpenoid from Endophytic Fungus *Periconia* sp. with Neural Anti-inflammatory Activity

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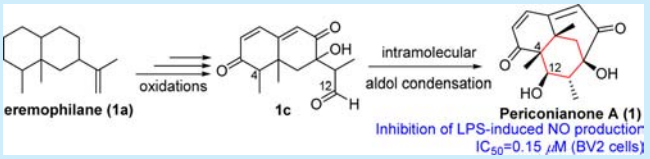
Supporting Information

ABSTRACT: Periconianone A (**1**), a polyoxygenated sesquiterpenoid with a new 6/6/6 tricarbocyclic skeleton, and periconianone B (**2**) were isolated from the endophytic fungus *Periconia* sp. Their structures and absolute configurations were elucidated by extensive spectroscopic analyses, calculated ECD, and single-crystal X-ray diffraction (Cu Kα). The biosynthesis of the unusual six-membered carbonic ring of **1** was postulated to be formed through intramolecular aldol condensation. Compounds **1** and **2** showed significant neural anti-inflammatory activity.



Endophytic fungi residing inside the normal tissues of host plants are an important source for the discovery of various structurally diverse secondary metabolites.[1] These naturally occurring compounds produced from endophytic fungi display a broad spectrum of biological functions, including uses as antitumor agents, antibiotics, and immunosuppressants.[1] Therefore, endophytic fungi have recently been attracting much more worldwide attention. As part of our ongoing search for structurally novel metabolites with interesting biological activities from endophytic fungi,[2] bioassay-guided fractionation of the EtOAc extract from the fermentation broth of the fungus *Periconia* sp. F-31 derived from the medicinal plant *Annonsa muricata* was systematically conducted. A variety of chromatographic methods led to the isolation of an unusual skeletal sesquiterpenoid, periconianone A (**1**), an eremophilane-type sesquiterpenoid, periconianone B (**2**) (Figure 1), and a known norsesquiterpenoid, dihydronaphthalene-2,6-dione (**3**).[3] Periconianone A (**1**) possesses a unique polyoxygenated rigid 6/6/6 carbocyclic skeleton consisting of one new six-membered carbonic ring formed through the linkage of C-4 and C-12. The structures and absolute configurations of **1** and **2** were established by extensive spectroscopic analyses, ECD calculations, and single-crystal X-ray diffraction. Herein, we describe their isolation, structural elucidation, plausible biogenetic pathway, and neural anti-inflammatory activity.

Periconianone A (**1**)[4] was obtained as colorless block crystals (cyclohexane–acetone). Its molecular formula was determined to be $C_{15}H_{18}O_4$ by HRESIMS at a *m*/*z* of 263.1271 $[M + H]^+$ (calcd for $C_{15}H_{19}O_4$, 263.1283). The IR spectrum showed the presence of hydroxyl (3453 and 3428 cm^{-1}) and conjugated carbonyl (1668 cm^{-1}) groups. The ^1H NMR spectrum of **1** (Table 1) revealed the presence of three olefinic protons at δ_H 7.45 (1H, dd, J = 10.2, 1.2 Hz), 6.12 (1H, s), and 5.94 (1H, d, J = 10.2 Hz); two methine protons at δ_H 3.32 (1H, dd, J = 10.2, 7.2 Hz) and 1.59 (1H, m); one methylene protons at δ_H 1.95 (1H, d, J = 13.8 Hz) and 1.73 (1H, d, J = 13.8 Hz); three methyl protons at δ_H 1.07 (3H, s), 1.05 (3H, s), and 0.75 (3H, d, J = 6.6 Hz); and two hydroxyls at δ_H 5.14 (1H, s) and 4.87 (1H, d, J = 7.2 Hz). The ^{13}C NMR and DEPT spectra showed 15 carbon resonances (Table 1), which consisted of six quaternary carbons (δ_C 199.9, 198.7, 161.4, 75.7, 56.0, and 44.8, including two carbonyl, one olefinic, and one oxygenated carbon), five methine carbons (δ_C 142.3, 128.8, 123.4, 72.8, and 44.8, including three olefinic and one oxygenated carbon), one methylene carbon (δ_C 40.2), and three methyl carbons (δ_C 23.3, 12.1, and 8.0). The HMBC correlations (Figure 2) of H-1/C-3, C-5, C-9, and C-10; H-2/C-4 and C-10; H_3-14/C-3, C-4, C-5, and C-12; H_2-6/C-4, C-7, C-8,

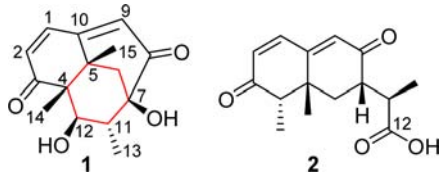


Figure 1. Chemical structures of **1** and **2**.

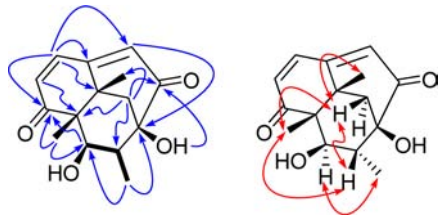


Figure 2. ^1H–^1H COSY (—), key HMBC (→), and NOESY (↔) correlations of **1**.

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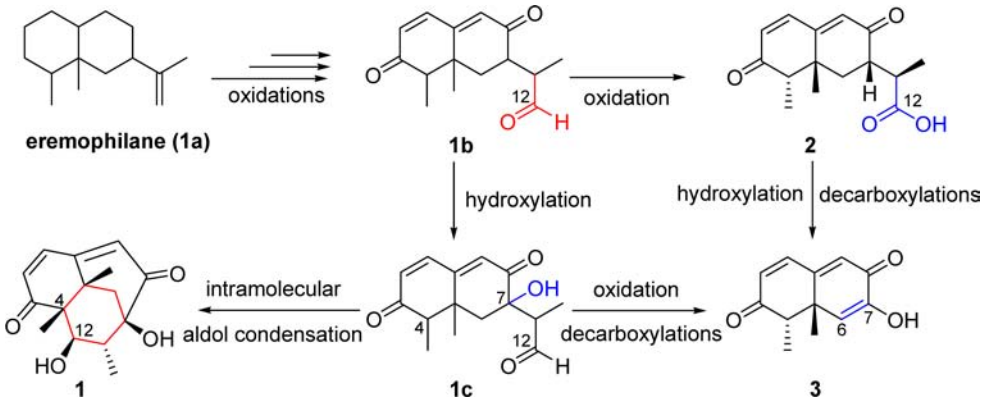
Table 1. ^{1}H and ^{13}C NMR Data of Periconianones A and B (1 and 2)[a]

	1		2	
no.	δ_C	δ_H[b]	δ_C	δ_H[b]
1	142.3 d	7.45 dd (10.2, 1.2)	142.6 d	7.28 d (9.6)
2	128.8 d	5.94 d (10.2)	129.1 d	6.12 d (9.6)
3	199.9 s		202.5 s	
4	56.0 s		52.4 d	2.37 q (7.2)
5	44.8 s		39.2 s	
6	40.2 t	1.95 d (13.8, Hβ)	32.5 t	2.11 dd (14.4, 13.2, Hα)
		1.73 d (13.8, Hα)		1.52 dd (13.2, 4.8, Hβ)
7	75.7 s		43.6 d	3.04 ddd (14.4, 4.8, 4.8)
8	198.7 s		198.6 s	
9	123.4 d	6.12 s	130.6 d	6.25 s
10	161.4 s		155.9 s	
11	44.8 d	1.59 m	37.6 d	2.91 dq (4.8, 7.2)
12	72.8 d	3.32 dd (10.2, 7.2)	176.4 s	
13	12.1 q	0.75 d (6.6)	13.0 q	1.02 d (7.2)
14	8.0 q	1.07 s	14.4 q	0.96 d (7.2)
15	23.3 q	1.05 s	24.6 q	1.28 s
7-OH		5.14 s		
12-OH		4.87 d (7.2)		12.19 s

[a] ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz), DMSO-d_6. [b] Multiplicities and coupling constants (J) in Hz are in parentheses.

Scheme 1. Hypothetical Biosynthetic Pathway of 1−3



C-10, C-11, and C-15; and H-9/C-1, C-5, and C-7 indicated the presence of a naphthalenedione moiety. Furthermore, the HMBC cross-peaks for H-11/C-6, C-7, C-8, C-12, and C-13; H-12/C-3, C-5, C-13, and C-14; as well as 12-OH/C-4, C-11, and C-12 confirmed another cyclohexane ring, which indicated a new sesquiterpenoid bearing an unusual rigid 6/6/6 tricyclic skeleton with C-4 and C-12 linked (Figure 1 and Scheme 1).

The relative configuration of **1** was established by NOE experiments (Figure 2) and the values of the coupling constants. The enhancement of H-12 when H_3-13 was irradiated suggested the *syn*-orientation of H-12 and H_3-13. The large coupling constant value of $J_{H\text{-}11,H\text{-}12}$ (10.2 Hz) supported that both H-11 and H-12 were located in an axial orientation and were on the opposite side. The NOEs of H-11/H-6β and H_3-14, and H-6β/H_3-14 and H_3-15 indicated the β-orientation of H-11, H_3-14, and H_3-15 (Supporting Information Figures S15 and S16). To prove the above assignments and determine the absolute configuration of **1**, a single-crystal X-ray diffraction pattern was obtained by the anomalous scattering of Cu Kα radiation (CCDC 975758). An ORTEP drawing with the atom numbering scheme indicated is shown in Figure 3, and it unambiguously demonstrates the absolute configuration for **1** as 4*R*,5*S*,7*R*,11*R*,12*R*.

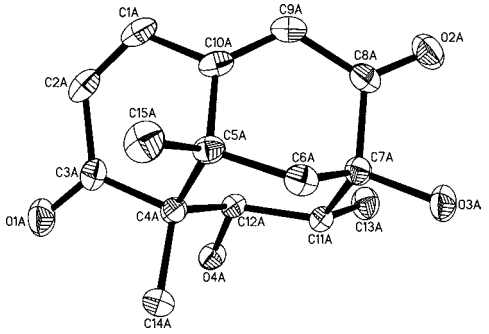


Figure 3. Structure of **1** resulting from single-crystal X-ray diffraction.

Periconianone B (**2**)[5] was isolated as a colorless gum (acetone), and it gave a HRESIMS ion peak at a m/z of 263.1280 $[M + H]^+$, which corresponded to a molecular formula of $C_{15}H_{18}O_4$. The ^{1}H and ^{13}C NMR spectroscopic analysis of **2** were similar to those of pleodendione[6] except for the absence of the methyl group (δ_H 0.82 and δ_C 20.4) and the presence of a carboxyl moiety (δ_H 12.19 and δ_C 176.4). The location of the carboxyl moiety was determined by the HMBC correlations of C-12/H-7, H-11, and H-13. The NOESY correlations of H_3-15/H-

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4, H-6β, and H-7 indicated their β-orientations, and the NOESY correlations of H-6α/H_3-14 established the α-orientations of H_3-14 (Figures S1 and S27).

The existence of an asymmetric diene in **2** as a chromophore can cause the Cotton effect. Therefore, the absolute configuration of the chiral center (C-5) in the nonplanar skeleton could be determined by the analysis of the helicity rule (see page S6 in the Supporting Information).[7] In the CD spectrum, the positive Cotton effect at 285.5 nm of **2** supported a 5*R* configuration (Figures S2 and S31), which established the 4*S*,5*R*,7*S* configuration of **2**. To determine the absolute configuration of C-11, ECD calculations of **2** were performed by the time-dependent density functional theory (TD-DFT) method at the TDDFT/B3LYP/aug-cc-pVDZ//B3LYP/6-31G(d,p) level in the gas phase (see pp S6−S7).[8] Comparison of the theoretically calculated and experimental ECD curves (Figures S5 and S6) led to the determination of a *R* configuration for C-11. Thus, the absolute configuration of **2** was assigned as 4*S*,5*R*,7*S*,11*R*.

To the best of our knowledge, periconianone A (**1**) represents the first example of a polyoxygenated sesquiterpenoid bearing this unusual 6/6/6 tricyclic ring system with the unusual carbonic ring linked by C-4 and C-12. The biosynthesis of **1**−**3** (Scheme 1) was proposed to originate from an eremophilane-type sesquiterpenoid (**1a**), which would then be transformed through a series of oxidations to **1b** featuring a 3,8-diketone and a 12-carbaldehyde. Further oxidation(s) would yield **2** and **1c**. Subsequently, the C-4−C-12 carbon bond formation via an intramolecular aldol condensation in **1c** would lead to the generation of an unusual six-membered carbonic ring, affording a rigid molecule, **1**. Compound **3** might originate from two routes, including from **1c** by oxidation and decarboxylations and from **2** by hydroxylation and decarboxylations.

Compounds **1**−**3** were tested for their neural anti-inflammatory activity using lipopolysaccharide (LPS)-induced NO production in mouse microglia BV2 cells as well as curcumin as a positive control. Microglia are endogenous immune cells in the central nervous system (CNS) that play critical roles in neurodegenerative disorders, such as Parkinson's disease (PD) and Alzheimer's disease (AD).[9] Compounds **1**−**3** exhibited significant inhibition of LPS-induced NO production with IC_{50} values of 0.15, 0.38, and 0.23 μM, respectively, making **1**−**3** much more potent than curcumin (IC_{50} = 3.9 μM). Thus, compounds **1**−**3** might be promising lead compounds for the treatment of PD and AD.[10]

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures; MS, IR, UV, 1D and 2D NMR, and CD spectra for **1**−**2**; ECD calculations of **2**; X-ray crystallographic data and cif. file for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(4) Periconianone A (**1**): colorless block crystals (cyclohexane−acetone); $[\alpha]_D^{20}$ +144.8 (*c* 0.17, MeOH); IR (ν_{max}) 3453, 3428, 1668, 1033, and 889 cm^{-1}; UV (MeOH) λ_{max} (log ε) 288.0 (0.85) nm; CD (MeOH) $\Delta\varepsilon$ (nm) −11.97 (223), +3.75 (287); ESIMS *m*/*z* 263.1 $[M + H]^+$; HRESIMS *m*/*z* 263.1271 $[M + H]^+$ (calcd for $C_{15}H_{19}O_4$, 263.1283); ^1H and ^{13}C NMR data, see Table 1.

(5) Periconianone B (**2**): colorless gum (acetone); $[\alpha]_D^{20}$ +24.0 (*c* 0.11, MeOH); IR (ν_{max}) 3304, 2974, 1718, 1668, 1254, and 1190 cm^{-1}; UV (MeOH) λ_{max} (log ε) 203 (0.22), 287 (0.75) nm; CD (MeOH) $\Delta\varepsilon$ (nm) −4.79 (208.5), +7.58 (285.5), −1.10 (354.5); ESIMS *m*/*z* 262.9 $[M + H]^+$, 285.0 $[M + Na]^+$; HRESIMS *m*/*z* 263.1280 $[M + H]^+$ (calcd for $C_{15}H_{19}O_4$, 263.1283); ^1H and ^{13}C NMR data, see Table 1.

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