Brocaeloids A–C, 4-Oxoquinoline and Indole Alkaloids with C-2 Reversed Prenylation from the Mangrove-Derived Endophytic Fungus *Penicillium brocae*

Peng Zhang,[a,b][‡] Ling-Hong Meng,[a,b][‡] Attila Mándi,[c] Tibor Kurtán,*[c] Xiao-Ming Li,[a] Yang Liu,[a,b] Xin Li,[a,b] Chun-Shun Li,[a] and Bin-Gui Wang*[a]

**Keywords:** Natural products / Alkaloids / Circular dichroism / Density functional calculations

Three new alkaloids, brocaeloids A–C (1–3), containing C-2 reversed prenylation, were isolated from cultures of *Penicillium brocae* MA-192, an endophytic fungus obtained from the fresh leaves of the marine mangrove plant *Avicennia marina*. Their structures were determined on the basis of 1D and 2D NMR spectroscopy as well as by high-resolution mass spectrometry. The absolute configuration of brocaeloid A (1) was established by gas-phase and solution conformational analysis and TDDFT-ECD calculations, which revealed that the fused hetero-ring adopted M-helicity conformation with axial orientation of the C-2 and C-3 substituents. The correct assignment of the hetero-ring conformation was found to be crucial in determining the relative and absolute configuration. Based on ECD calculations, the helicity of the 2,3-dihydroquinoline-4(1H)-one chromophore was correlated with the characteristic ECD transitions, and the resultant helicity rule was found to coincide with that of the chroman-4-one chromophore. X-ray single-crystal analysis of 1 by Cu-Kα radiation also confirmed the result of the stereochemical analysis obtained from ECD calculations. Brocaeloid B (2) showed lethality against brine shrimp (*Artemia salina*) with an LD$_{50}$ value of 36.7 µM.

**Introduction**

Marine-derived fungi are an important source of secondary metabolites that can possess both unique structure and potent pharmaceutical activity.[1] In recent years, an increasing number of bioactive natural products have been isolated from fungi associated with mangrove plants and their rhizospheric soils.[2] Our previous investigation of mangrove-derived endophytic fungi has resulted in the isolation and identification of a number of structurally unique and biological active secondary metabolites.[3–7] As a continuation of our investigations on the characterization of new bioactive secondary metabolites from marine-derived endophytes, three new alkaloids, brocaeloids A–C (1–3), which contain C-2-reversed prenylation in the molecules (Figure 1), were isolated and identified from *Penicillium brocae* MA-192, an endophytic fungus obtained from the fresh leaves of marine mangrove plant *Avicennia marina*. Herein, we report the isolation, structure elucidation, absolute configuration assignment, and biological activity of these compounds.

**Results and Discussion**

The ethyl acetate (EtOAc) soluble extract of *P. brocae* MA-192 was subjected to silica gel vacuum liquid chromatography (VLC) and was further purified by a com-
Combination of column chromatography (CC) on silica gel, Sephadex LH-20, Lobar LiChroprep RP-18, and semi-preparative HPLC to furnish three new compounds 1–3.

Compound 1 was initially obtained as a light-yellow amorphous solid. Its molecular formula C_{17}H_{22}O_{2}N_{2} was established on the basis of the HRMS (ESI) ion at m/z 287.1755 [M + H]^{+} (calcld. for C_{17}H_{22}O_{2}N_{2}{^{2+}}, 287.1754), indicating eight degrees of unsaturation. The 1H NMR spectrum (Table 1), four aromatic signals resonating at \( \delta_{H} = 7.53 \) (d, \( J = 7.8 \) Hz, 5-H), 6.51 (t, \( J = 7.8 \) Hz, 6-H), 7.24 (t, \( J = 7.8 \) Hz, 7-H), and 6.81 ppm (d, \( J = 7.8 \) Hz, 8-H), indicated the presence of a 1,2-disubstituted benzene system, which was supported by the corresponding COSY correlations as shown in Figure 2. A total of 17 carbon atoms including three methyls, two methylenes (one nitrogenated sp^{3} and one terminal sp^{3}), seven methines (one nitrogenated sp^{3} and five sp^{2}), and five quaternary (one amide and one ketone) carbon signals were observed in the \(^{13}\)C NMR and DEPT spectra (Table 1). The complete NMR assignments and connectivity of 1 were further determined by analysis of the 2D NMR spectroscopic data. The observed COSY correlations between N1-H and 2-H and between 10-H and CH_{2}-11 in the COSY spectrum, as well as HMBC correlations from the methyl protons CH_{3}-12 and CH_{3}-13 to C-2, C-9, and C-10 (Figure 2) indicated the presence of a \(-\text{NHCHC}(\text{CH})_{3}\text{CH}=\text{CH}_{2}\)-fragment in the structure of 1. The clear HMBC cross-peaks from 2-H to the nitrogenated aromatic carbon C-8a revealed that the above fragment should connect to the benzene ring through NH-1 at C-8a. Additionally, COSY correlations between NH-15 to CH_{2}-14 and between CH_{2}-14 to 3-H as well as the HMBC correlation from 3-H to the ketone carbonyl group C-4 established the fragment COCHCH_{2}NH-. The HMBC correlation from the aromatic proton 5-H to the C-4 ketone carbonyl established the connection of this fragment to C-4a. The HMBC cross-peak from the acetyl methyl protons CH_{3}-17 to the carbonyl group C-16 implied the existence of an acetyl group. Combined with the characteristic signals, the acetyl group was deduced to be connected with N-15 to form the acetamide moiety. The fragments deduced above including a benzene ring, a double bond, and two carbonyl groups accounted seven out of eight degrees of unsaturation, suggesting the presence of an additional fused 2,3-dihydropyridin ring in 1. The COSY correlation between 2-H and 3-H supported the presence of a 2,3-dihydroquinoline-4(1H)-one moiety, which was further confirmed by the HMBC correlations from 2-H to C-3, C-4, and C-14 and from CH_{2}-14 to C-2. The planar structure of 1 was thus established as shown in Figure 1.

![Figure 2](image-url)

Figure 2. Key COSY (bold line) and HMBC (arrow) correlations of 1–3.

Compound 1 has two chirality centers, implying four possible stereoisomers, the number of which can be reduced to two by determining the relative configuration. The 2-H and 3-H protons showed a small \(^{3}J_{H,H}\) coupling constant.

**Table 1.** \(^{1}\)H and \(^{13}\)C NMR data (500 and 125 MHz, resp.) of 1–3. Assignments were corroborated by 2D NMR spectroscopy.

<table>
<thead>
<tr>
<th>Position</th>
<th>1 (measured in [D_{6}]acetone) (\delta_{H}) (mult., (J) in Hz)</th>
<th>(\delta_{C})</th>
<th>2 (measured in CDCl_{3}) (\delta_{H}) (mult., (J) in Hz)</th>
<th>(\delta_{C})</th>
<th>3 (measured in CDCl_{3}) (\delta_{H}) (mult., (J) in Hz)</th>
<th>(\delta_{C})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-NH</td>
<td>6.24 (br. s) (\delta_{H} = 7.53) (d, (J = 7.8) Hz, 5-H)</td>
<td>11.64 (br. s)</td>
<td>175.9</td>
<td>7.94 (br. s) (\delta_{H} = 7.53) (d, (J = 7.8) Hz, 5-H)</td>
<td>140.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.32 (d, 3.9)</td>
<td>60.9</td>
<td>39.5</td>
<td>3.08 (t, 7.0)</td>
<td>25.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.80 (ddd, 7.6, 6.5, 3.9)</td>
<td>46.8</td>
<td>3.26 (t, 5.8)</td>
<td>203.3</td>
<td>108.1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>193.4</td>
<td>116.4</td>
<td>121.8</td>
<td>129.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>101.5</td>
<td>114.3</td>
<td>121.0</td>
<td>110.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.53 (d, 7.8)</td>
<td>126.4</td>
<td>7.87 (d, 8.2)</td>
<td>130.7</td>
<td>7.57 (d, 7.4)</td>
<td>118.4</td>
</tr>
<tr>
<td>6</td>
<td>6.51 (t, 7.8)</td>
<td>115.4</td>
<td>7.09 (t, 8.2)</td>
<td>122.4</td>
<td>7.11 (t, 7.4)</td>
<td>119.6</td>
</tr>
<tr>
<td>7</td>
<td>7.24 (t, 7.8)</td>
<td>135.0</td>
<td>7.54 (t, 8.2)</td>
<td>135.2</td>
<td>7.16 (t, 7.4)</td>
<td>121.7</td>
</tr>
<tr>
<td>8</td>
<td>6.81 (d, 7.8)</td>
<td>114.3</td>
<td>8.75 (d, 8.2)</td>
<td>121.0</td>
<td>7.32 (d, 7.4)</td>
<td>110.7</td>
</tr>
<tr>
<td>8a</td>
<td>150.5</td>
<td>42.9</td>
<td>46.8</td>
<td>39.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>105.5</td>
<td>141.1</td>
<td>134.3</td>
<td>39.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5.74 (dd, 17.5, 10.8)</td>
<td>144.8</td>
<td>6.13 (dd, 17.3, 10.6)</td>
<td>142.6</td>
<td>6.14 (dd, 17.6, 10.4)</td>
<td>146.1</td>
</tr>
<tr>
<td>11</td>
<td>4.95 (d, 17.5)</td>
<td>112.5</td>
<td>5.32 (d, 17.3)</td>
<td>114.6</td>
<td>5.18 (d, 10.4)</td>
<td>112.3</td>
</tr>
<tr>
<td>12</td>
<td>4.93 (d, 10.8)</td>
<td>5.29 (d, 10.6)</td>
<td>5.17 (d, 17.6)</td>
<td>28.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>1.01 (s)</td>
<td>23.8</td>
<td>1.42 (s)</td>
<td>24.7</td>
<td>1.57 (s)</td>
<td>28.0</td>
</tr>
<tr>
<td>14</td>
<td>0.95 (s)</td>
<td>22.3</td>
<td>1.42 (s)</td>
<td>24.7</td>
<td>1.57 (s)</td>
<td>28.0</td>
</tr>
<tr>
<td>15</td>
<td>3.47 (dd, 13.5, 7.6)</td>
<td>41.4</td>
<td>3.63 (t, 5.8)</td>
<td>34.6</td>
<td>3.55 (t, 7.0)</td>
<td>40.6</td>
</tr>
<tr>
<td>16</td>
<td>3.21 (dd, 13.5, 6.5)</td>
<td>7.17 (br. s)</td>
<td>6.10 (br. s)</td>
<td>5.59 (br. s)</td>
<td>170.4</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>1.87 (s)</td>
<td>21.9</td>
<td>1.96 (s)</td>
<td>23.3</td>
<td>1.93 (s)</td>
<td>23.5</td>
</tr>
</tbody>
</table>
TDDFT-ECD calculations were therefore carried out. This previously by X-ray diffraction analysis, details of the conformational configuration of trans-(2R,3S)-1, with equatorial and axial orientation of the two methine protons (structure III and IV). Thus the relative configuration could not be determined unambiguously on the basis of the $J_{1H,1H}$ and NOE data. For the configurational assignment of I, conformational analysis of the arbitrarily chosen trans-(2R,3S)-1 and cis-(2S,3S)-1 in the gas phase and with polarizable continuum model (PCM) for acetonitrile and their TDDFT-ECD calculations were therefore carried out. This approach allowed the relative configuration of I to be determined as trans, and the absolute configuration as (2S,3R).

Because in the late stage of our stereochemical study, the relative configuration of I was also determined unambiguously by X-ray diffraction analysis, details of the conformational analysis and TDDFT-ECD calculation of cis-(2S,3S)-1, performed to identify the relative configuration by ECD study, is presented only in the Supporting Information (Figures S1–S4). With the configuration established by X-ray analysis, this part of the investigation may be viewed as a case study in determining the relative configuration and preferred conformation by ECD analysis for a relatively simple molecule, the NMR analysis of which was not suitable for that purpose independently.

The gas-phase B3LYP/6-31G(d) reoptimization of the initial MMFF conformers of trans-(2R,3S)-1 afforded nine conformers above 2% population (Figure S5). The two lowest-energy conformers (20.5 and 16.6% populations) had a hetero-ring of $M$-helicity with axial 2-H and 3-H (structure I in Figure 3). The $M$-helicity form had a total population of 47.0% represented by four conformers. The $P$-helicity form with equatorial 2-H and 3-H (structure II in Figure 3) had a comparable 36.2% overall population deriving from four conformers, which differed in the orientation of the C-2 and C-3 substituents. The ECD spectrum of I in acetonitrile showed a broad intense positive Cotton effect (CE) at 384 nm, negative CEs below 323 and 240 nm, and positive CEs at 213 nm and below 205 nm (see Exp. Section). The B3LYP/TZVP computed ECD spectra of the $P$-helicity conformers (e.g., conformer C) gave an intense long-wavelength CE corresponding to the 384 nm experimental ECD band (Figure 4), whereas the $M$-helicity conformers (e.g., conformer A) showed completely different ECD curves. The Boltzmann-weighted computed ECD spectra of the gas-phase B3LYP/6-31G(d) conformers of trans-(2R,3S)-1 showed a mirror image curve of the experimental ECD spectrum with acceptable agreement with the tested three methods (B3LYP/TZVP shown in Figure 4).

Figure 3. (a) Equilibrating $P$- and $M$-helicity conformers of trans-(2R,3S)-1 as viewed from the direction of the fused benzene ring (upper) and as obtained by gas phase B3LYP/6-31G(d) and solution (PCM model for acetonitrile) B97D/TZVP conformational analysis (lower). (b) Equilibrating $P$- and $M$-helicity conformers of cis-(2S,3S)-1 as viewed from the direction of the fused benzene ring (upper) and as obtained by solution (PCM model for acetonitrile) B97D/TZVP conformational analysis (lower).

Figure 4. Solution ECD spectrum of I in acetonitrile (black) compared with the Boltzmann-weighted B3LYP/TZVP (red, average of nine conformers) computed ECD spectra of the gas-phase B3LYP/6-31G(d) conformers of trans-(2R,3S)-1. Rotational strengths of conformer A ($M$-helicity, red bars) and C ($P$-helicity, orange bar) are shown to emphasize differences in their ECD spectra.

The conformational analysis of trans-(2R,3S)-1 was repeated at the B97D/TZVP level with the PCM model for
Figure 5. Structures and populations of conformers obtained by B97D/TZVP reoptimization with PCM solvent model for acetonitrile of the initial MMFF conformers of trans-(2R,3S)-1 above 2% population.

acetonitrile, which resulted in 13 conformers (65.7% total population above 2%) corresponding to the \( P \)-helicity form with \textit{equatorial} 2-H and 3-H (structure II in Figure 3). In all the conformers, the hetero-ring adopted a half-chair conformation, which is indicated by the similar values of the torsion angles \( \omega_{C-4a,C-8a,N-1,C-2} \) and \( \omega_{C-8a,C-4a,C-4,C-3} \) (–14.2° and –12.1°, respectively for conformer A). All the conformers had \( P \)-helicity and they differed in the rotation of the atoms or groups of the C-2 and C-3 substituents. In contrast to the gas-phase calculation, and in accordance with the NMR spectroscopic data, this method clearly predicted the prevalence of the 2-H\textit{eq}, 3-H\textit{eq} conformer (Figure 5), which is attributed to the presence of the bulky 2-methylbut-3-en-2-yl substituent. The computed ECD spectra of the B97D/TZVP solution conformers of trans-(2R,3S)-1 were consistently mirror image of the experimental ECD spectrum with the three tested methods (B3LYP/TZVP shown in Figure 6). This proved that the absolute configuration of 1 is (2S,3R) and it was named brocaeloid A.

The 2,3-dihydroquinoline-4(1H)-one chromophore of brocaeloid A (1) belongs to a group of cyclic aryl ketones that includes the chromane-4-one chromophore in flavanones\textsuperscript{[8]} 2-hydroxyflavanones,\textsuperscript{[8]} 2-alkylchromanones,\textsuperscript{[9]} and isoflavanones (Figure 7).\textsuperscript{[10,11]} The sign of the high-wavelength \( n-\pi^* \) CE of the latter was correlated with the helicity of their hetero-ring by helicity rules, according to which \( P \)-helicity of the hetero-ring adopting envelope or half-chair conformation is manifested in a positive \( n-\pi^* \) CE above 300 nm.\textsuperscript{[12]} Because the 2,3-dihydroquinoline-4(1H)-one chromophore is the nitrogen analogue of the chromane-4-one chromophore, a similar helicity rule is expected between the sign of the \( n-\pi^* \) CE and the helicity of the hetero-ring of the dihydroquinoline-4(1H)-one moiety. Our conformational analysis revealed that the hetero-ring of brocaeloid A has half-chair conformation with \( M \) helicity, which should result in negative \( n-\pi^* \) CE. In contrast, the highest-wavelength ECD band of 1 had a positive CE at 384 nm. Analysis of the Kohn–Sham orbitals showed that the 384 nm UV transition is a pure HOMO–LUMO transition of \( \pi-\pi^* \) origin, whereas the \( n-\pi^* \) transition belongs to the
Brocaeloids A–C Alkaloids with C-2 Reversed Prenylation

Figure 7. Correlation between the helicity of the hetero-ring in (S)-flavanone, (2R,3R)-3-hydroxyflavanone, (R)-2-methylchroman-4-one, (R)-isoflavanone, and brocaeloid A (1) and the sign of the n-π* CE.

![Chemical Structures](Image)

Figure 8. Kohn–Sham orbitals of brocaeloid A (1) responsible for the 384 nm π-π* (HOMO→LUMO) and 323 nm n-π* (HOMO→3→LUMO and HOMO→2→LUMO) transitions extracted from B3LYP/TZVP calculation of the lowest-energy B97D/TZVP (PCM model for acetonitrile) solution conformer and plotted with an isovalue of 0.032: (a) HOMO, (b) LUMO, (c) HOMO-3, and (d) HOMO-2.

![ECD Calculations](Image)

Figure 9. X-ray crystal structure of 1 (a different numbering system is used for the structure in the text).

![Crystal Structure](Image)

323 nm band with negative CE (Figure 8). Thus, M-helicity of brocaeloid A (1) with (2S,3R) absolute configuration results in a positive π-π* and a negative n-π* CE, which parallels the helicity rule of the chromane-4-one chromophore.

Simultaneously with the ECD calculation studies, crystallization of 1 was performed. Although compound 1 was initially obtained as a light-yellow amorphous solid, after many attempts, single crystals that were suitable for X-ray analysis were obtained by slow evaporation of a solution of 1 in MeOH/CHCl₃ (1:1). The results of the X-ray diffraction analysis confirmed independently that brocaeloid A (1) indeed has trans relative configuration with axial orientation of the C-2 and C-3 substituents (Figure 9).

Compound 2, a colorless oil, was determined to have the molecular formula C₁₇H₂₂O₂N₂ on the basis of HRMS (ESI), suggesting eight degrees of unsaturation. The ¹H and ¹³C NMR spectroscopic data of 2 (Table 1) were very similar to those of 1, except that the two methines at δ_C = 60.9 (C-2) and 46.8 ppm (C-3) of 1 were not present in the spectrum of 2. Instead, signals for an additional carbonyl at δ_C = 175.9 ppm (C-2) and a methylene group at δ_C = 39.5 ppm (C-3) were observed in the NMR spectra of 2. The above data implied that the hexatomic ring in 1 was opened, and one carbon atom, either C-2 or C-3, was oxygenated to a carbonyl in 2. The COSY correlation between the nitrogenated methylene (CH₂-14) and the newly presented methylene (CH₂-3), as well as the HMBC correlations from the gem-dimethyl groups CH₃-12 and CH₃-13 to the carbonyl carbon C-2 supported the above deduction, and the oxygenated C-2 was also established to be a carbonyl carbon. The structure of compound 2 was thus determined and named brocaeloid B.

Compound 3 was obtained as a colorless oil and the molecular formula C₁₇H₂₂O₃N₂ was determined by analysis of the HRMS (ESI). Detailed comparison of the NMR spectroscopic data of 3 with those of N₃-acetyltryptamine (Figure 1), an indole alkaloid isolated from an unidentified marine algicoloic fungus, suggested that they shared similar structure features. However, compound 3 contained an additional isoprenyl substitution at C-2. The significant differ-
Scheme 1. Plausible biosynthetic pathways for compounds 1–3.

Enzymes in the NMR spectrum of 3 were the absence of an olefinic proton signal for 2-H, which was observed at δ_H = 7.04 ppm (d, J = 2.2 Hz) in the 1H NMR spectrum of N-acetyltryptamine,[13] and the presence of the signals for a trans-isopentene group (C-9–C-13). The detected HMBC correlations from the two gem-methyl groups of the isopentene (CH₃-12 and CH₃-13) to C-2 verified the prenylation of the indole moiety at C-2. Accordingly, the structure of 3 was determined, and the trivial name brocaeloid C was assigned.

Compounds containing N-acetyl groups were described as naturally occurring microbial secondary metabolites in previous reports.[13,14] The close resemblance of compounds 1–3 indicated that they are probably generated by a common biosynthesis pathway from a tryptophan precursor (Scheme 1).[15–18]

Compounds 1–3 were evaluated for the lethality against brine shrimp (Artemia salina), for DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging potency, and for antibacterial activity against five bacteria. None of these compounds showed DPPH radical scavenging or antibacterial activity. However, compound 2 exhibited potent lethal activity with an LD₅₀ value of 36.7 µg/mL, which is more active than that of the positive control colchicine (with LD₅₀ 87.6 µg/mL). The other tested compounds displayed either weak or no activity (LD₅₀ > 100 µg/mL).

Conclusions

Three new alkaloids, brocaeloids A–C (1–3), containing reversed prenylation in their structures, were isolated from cultures of marine-derived Penicillium brocae MA-192. The structures of 1–3 were elucidated by analyzing spectroscopic data generated by 1D and 2D NMR and mass spectrometry. The absolute configuration of brocaeloid A (1) was established by the TDDFT-ECD calculation of its cis- and trans-isomers. Calculations showed the importance of conformation in determining relative and absolute configuration, because 1 exists in solution and solid-state as the trans-diaxial conformer with M-helicity due to the steric hindrance of the C-2 and C-3 substituents. Compound 2 showed lethality against brine shrimp (Artemia salina) with an LD₅₀ value of 36.7 µg/mL.

Experimental Section

General: Optical rotations were determined with an Optical Activity AA-55 digital polarimeter. UV Spectra were measured with a Lenguang-Gold-Spectrulab-54 UV/Vis spectrophotometer, λ_max (log ε) in nm. ECD spectra were recorded with a J-810 spectropolarimeter with a mm cell using 1 nm bandwidth, 2 s response, standard sensitivity, 100 nm/min scanning speed and 3 accumulations. NMR spectra were recorded with a Bruker–Avance-500 spectrometer (500 MHz for 1H and 125 MHz for 13C), δ in ppm, J in Hz. Low- and high-resolution ESI-MS were acquired with a VG-Autospec-3000 mass spectrometer. Semipreparative HPLC were performed with a Dionex HPLC system equipped with a P680 pump, an ASI-100 automated sample injector, and an UVD340U multiple wavelength detector (Sinochrom ODS-BP column, 10 × 300 mm, 10 µm, flow rate 3 mL/min). Column chromatography (CC) experiments were accomplished by using commercial silica gel (SiO₂; 200–300 mesh; Qingdao Haiyang Chemical Group Co.), Lobar LiChroprep RP-18 (40–63 µm; Merck), and Sephadex LH-20 (Pharmacia). TLC analyses were performed using precoated SiO₂ GF-254 plates (Qingdao Haiyang Chemical Group Co.). All solvents used for extraction and purification were distilled prior to use.
Brocaloids A–C Alkaloids with C-2 Reversed Prenylation

Fungal Strain: The endophytic fungus *Penicillium brocae* MA-192 was isolated from fresh leaves of the marine mangrove plant *Avicennia marina*, which was collected from Hainan island, P. R. China, in August 2012. The fungus was identified by analysis of its ITS region of the rDNA, as described in our previous report.[19] The sequence derived from the fungal strain was deposited at GenBank, with accession No. KF513181. A BLAST search result showed that the sequence was the most similar (99%) to the sequence of *Penicillium brocae* (compared to AF484394). The strain is preserved at the Institute of Oceanology, Chinese Academy of Sciences.

Fermentation: For chemical investigations, the fungal strain was statically fermented in a 1000-mL Erlenmeyer flask containing 300 mL of the PDB medium (potato dextrose broth: 2% mannitol, 1% glucose, 0.3% peptone, 0.5% yeast extract, and seawater added up to 300 mL, pH 6.5–7.0, adjusted with 10% NaOH/flask, 60 flasks) at room temperature for 30 d.

Extraction and Isolation: The mycelium and broth were separated by filtration. The mycelium were homogenized with a waring blender, and the mycelium and broth were exhaustively extracted with EtOAc to give a crude extract (28.0 g), which was dried and fractionated by silica gel vacuum liquid chromatography (SILC) using solvents of increasing polarity from petroleum ether (PE) to MeOH to yield eight fractions (Fr. 1–8) based on TLC analysis. Fr. 6 (6.3 g), eluted with petroleum ether/EtOAc (1:1), was further separated by CC (SiO$_2$/CHCl$_3$/MeOH, 40:1 to 10:1; Lobar LiChro-prep RP-18; MeOH/H$_2$O, 3:7 to 0:1; Sephadex LH-20, MeOH), and finally preparative HPLC (MeOH/H$_2$O, 55%) to yield 3 (5.0 mg, Fr$_r$ = 30.51 min). Fr. 7 (3.4 g), eluted with CHCl$_3$/MeOH (20:1), was subjected to CC (SiO$_2$/CHCl$_3$/MeOH, 50:1 to 10:1; then Sephadex LH-20, MeOH) to yield 1 (7.0 mg) and 2 (15.8 mg).

Brocaloid A (1): Yellow prismatic crystals; m. p. 196–197 °C; [α]$_D^2$ = +225 (c = 0.20, MeOH). UV (MeOH): 238 (4.20), 262 (3.74), 395 (3.41) nm. ECD (MeCN, λ (Δε) = 5.83 × 10$^{-4}$m$^{-1}$c$^{-1}$) = 384 (+2.72), 323 (-0.55), 312 sh (-0.47), 273 (-0.21), 260 sh (-0.58), 240 (-3.71), 213 (+1.15) nm; positive below 205 nm. HRMS (ESI): [M + H]$^+$ calcd. for C$_{37}$H$_{32}$O$_6$N$_2$: m/z = 599.2115 (+0.7 mm, F(I00) = 0.0000). The lethality assay against brine shrimp was carried out by using the methods of the Ministry of Science and Technology of China (grant numbers 2013AA092901 and 2010CB83800) and from the National Natural Science Foundation of China (NSFC) (grant numbers 31270403 and 30910103914) is gratefully acknowledged. T.K. thanks the Hungarian National Research Foundation (OTKA K105871) for financial support and the National Information Infrastructure Development Institute (NIIFI), 10038 for CPU time.

Acknowledgments

Financial support by programs from the Ministry of Science and Technology of China (grant numbers 2013AA092901 and 2010CB83800) and from the National Natural Science Foundation of China (NSFC) (grant numbers 31270403 and 30910103914) is gratefully acknowledged. T.K. thanks the Hungarian National Research Foundation (OTKA K105871) for financial support and the National Information Infrastructure Development Institute (NIIFI), 10038 for CPU time.


29. CCDC-980111 for 1 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.


Received: January 15, 2014
Brocaeloids A–C, 4-Oxoquinoline and Indole Alkaloids with C-2 Reversed Prenylation from the Mangrove-Derived Endophytic Fungus *Penicillium brocae*.

Keywords: Natural products / Alkaloids / Circular dichroism / Density functional calculations