Structure and absolute configuration of penicilliumine, a new alkaloid from *Penicillium commune* 366606

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**Abstract**

Penicilliumine (1), a new structure was isolated from the fermentation of *Penicillium commune* 366606, a marine-derived fungus isolated from the sea water collected at Qingdao, China. HPLC chiral separation of 1 afforded two enantiomers (–)–penicilliumine and (+)-penicilliumine, respectively. The structure of 1 was established by comprehensive spectroscopic data, and single-crystal X-ray diffraction. The absolute configuration of enantiomers was determined by quantum mechanical computation of electronic circular dichroism (ECD).

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**Introduction**

Marine microorganisms are widely recognized as important sources of structurally and biologically novel natural products. For example, pyrolnitrin was isolated from *Pseudomonas bromoutilis* by Burckholder, unveiling the study of marine microorganism metabolites, and now it becomes a research hot-spot. As part of our ongoing investigation to discover structurally novel and bio-active natural compounds from marine fungi, a fungi Q-4, identified as *Penicillium commune* 366606, was isolated from water collected in Qingdao, China. Indeed, many metabolites, including new bioactive compounds have been obtained from *Penicillium*. In our recent efforts, we isolated an unusual alkaloid, penicilliumine (1), from the genus of *Penicillium commune* 366606. Its planar structure was well established by 2D NMR, such as HMBC and HSQC. Single-crystal X-ray diffraction using anomalous scattering of CuKα radiation, also indicated that the obtained 1 is a racemic mixture, HPLC separation of 1 on a chiral column afforded two individual enantiomers, (–)-penicilliumine and (+)-penicilliumine. Its absolute configuration (AC) was well established by using matrix method and ECD.

**Results and discussion**

Compound (1) (Fig. 1) was obtained as colorless crystal from MeOH. Its molecular formula was established as C_{20}H_{22}N_{2}O_{5} by HR-ESI-MS (found m/z 396.1432 [M], calcd 396.1434 for C_{20}H_{22}N_{2}O_{5}), requiring thirteen degrees of unsaturations. The IR displayed the existence of hydroxyl or amino group (3410 cm⁻¹), the strong absorption at (1669 cm⁻¹) suggested the presence of amide groups.

**1H NMR spectrum data of 1** (Table 1, dissolved in DMSO-d6) revealed the presence of two single methyl [δ_H 1.48 (3H, s, H-5'), 1.68 (3H, s, H-6')], combined with HSQC analyses revealed the presence of six active hydrogens at δ_H 12.22, 11.26, 8.18, 7.65, 6.27, and 5.89 (each 1H, s). The 13C NMR and DEPT spectroscopic data of 1 (Table 1) indicated 20 carbon resonances, including two methyl groups, eight methines and 10 quaternary carbons (three amide carbonyl groups, and two high-field ones). The 1H–1H COSY correlations (Fig. 2) of H-3 ([δ_H 8.45]/H-4[δ_H 7.45], H-4/H-5 ([δ_H 7.11], H-5/H-6/δ_H 7.72) and H-6'/[δ_H 8.11]) /H-7'/[δ_H 7.50], H-7'/H-8'/[δ_H 7.79], H-8'/H-9'/[δ_H 7.64], displayed the key spin systems. Moreover, from the HMBC spectrum (Fig. 2), two methyl groups could be located at C-3' and C-4', on the basis of the HMBC correlations of H-6' to C-4', C-2', C-3' and H-5' to C-3', C-4', C-2'. The correlations of H-1' with C-2', C-2', suggested the link of C-2', C-3', C-4', C-5'.
and the substitution position of the benzene ring. The correlation of H-6 with C-7, showed the position of the amide group. The correlations of H-3 with C-4, H-6' with C-10, H-7 with C-5' indicated the linkage of C-4', C-5', C-6' and C-10'. Thus, the planar structure of 1 was assigned as depicted, a new alkaloid, named as penicilliumine.

The relative configuration of 1 was finally confirmed by single-crystal X-ray diffraction (Fig. 3) using Cu(K\(\nu\)) radiation. The crystal of compound 1 is in the space group C2/c, which indicated that compound 1 is a racemic mixture, comprised of equal amounts of left- and right-handed enantiomers. Subsequent HPLC separation of 1 on a chiral column afforded two individual enantiomers (−)-1 and (+)-1. However, after the preparation of (−)-1 and (+)-1, no crystal was obtained.

Stereochemistry of 1 was studied. The two stereogenic centers are located on the chain instead of on the ring. As one of the typical acyclic chiral compounds, its stable conformations are generally more than the corresponding cyclic chiral compounds. In this case, the matrix method could be one of the choices for its AC study. Matrix method is a kind of a mathematic tool that majorly focuses on the energy of the system (wave function system). However, as mentioned above, if a chiral compound has many stable low energy conformations, it is difficult to investigate all stable conformations that may further be used in optical rotation calculations. In a new model construction, it is important to avoid the energy consideration in AC assignment. We have to focus on another characteristic of light, particle function that obeys the quantum statistics rules. It has no relationship with conformational energy. Matrix model is suggested for application for AC determination using optical rotation. It focuses on the different contribution of four substituents on optical rotation. Four independent parameters, such as comprehensive mass (\(m\)), radius (\(r\)), electronegativity (\(\chi\)), and symmetry (\(s\)) of the substituents were considered in the matrix. It is found that the optical rotation is proportional to the determinant of the matrix, namely, \([\chi]D = k_0 \times \text{det}(D)\). Therefore, the same series of chiral compounds will have the same \(k_0\) constant. However, due to the errors of measurement using different equipment and other factors, \(k_0\) will tend to be a constant. The \(\text{det}(D)\) was computed for (3',5',4')-R. The computed \(\text{det}(D)\) was −1.47 with \(k_0 = 2.61\) when its optical rotation of −30 was used. When (3',5',4')-S was used in \(\text{det}(D)\) computations, the \(k_0\) was 4.37, which is also located in the reliable range (mostly from 0.5 to 6.0). Therefore, its absolute configuration for (−)-1 should be either (3',5',4') or (3',5',4'). Since the relative configuration for 1 was well assigned by X-ray and ROSEY experiments as (3',5',4') or (3',4',5'). Therefore, (−)-1 should be (3',5',4').

Furthermore, to confirm the conclusion from matrix model, the electronic circular dichroism (ECD) for (3',R,4'S) was computed at the B3LYP/6-311+G(2d,p)//B3LYP/6-311+G(d) level in the gas phase and in solution using IFPCM model, respectively. After UV correction, the computed ECD and the experimental ECD are put together for comparison (Fig. 4). The predicted ECD of (3',R,4'S)

Figure 1. Structures of (±)-penicilliumine.

Table 1

<table>
<thead>
<tr>
<th>Position</th>
<th>(\delta_{1H}) multi ((j) in Hz)</th>
<th>(\delta_{13C}) multi ((j) in Hz)</th>
<th>(\delta_{13C}) ((j) in ppm)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>1.21 (6')</td>
<td>1.68 (3H, s)</td>
<td>22.1</td>
</tr>
<tr>
<td>2</td>
<td>1.38 (2')</td>
<td>1.16 (1H, s)</td>
<td>160.2</td>
</tr>
<tr>
<td>3</td>
<td>8.45 (1H, d, 8.3)</td>
<td>12.82 (1H, d, 7.8)</td>
<td>121.2</td>
</tr>
<tr>
<td>4</td>
<td>7.45 (1H, t, 7.3)</td>
<td>131.4 (4H, 1H, t, 7.3)</td>
<td>121.0</td>
</tr>
<tr>
<td>5</td>
<td>7.11 (1H, t, 7.3)</td>
<td>122.7 (5H, 1H, t, 7.3)</td>
<td>122.7</td>
</tr>
<tr>
<td>6</td>
<td>7.85 (1H, d, 7.8)</td>
<td>128.6 (6H, 1H, d, 7.8)</td>
<td>128.6</td>
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<tr>
<td>7</td>
<td>170.3 (7H, 1H, t, 7.3)</td>
<td>7.91 (1H, t, 7.3)</td>
<td>134.6</td>
</tr>
<tr>
<td>7-NH₂</td>
<td>8.18 (1H, s)</td>
<td>8.60 (1H, s)</td>
<td>126.7</td>
</tr>
<tr>
<td>7-NH₂</td>
<td>7.65 (1H, s)</td>
<td>7.64 (1H, d, 7.8)</td>
<td>127.2</td>
</tr>
<tr>
<td>1'</td>
<td>12.22 (1H, s)</td>
<td>9.6'</td>
<td>10.1</td>
</tr>
<tr>
<td>2'</td>
<td>174.6 (10')</td>
<td>147.7</td>
<td>147.7</td>
</tr>
<tr>
<td>3'</td>
<td>79.0 (5'H, 1H, s)</td>
<td>6.27 (1H, s)</td>
<td>147.7</td>
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<td>4'</td>
<td>77.9 (6'H, 1H, s)</td>
<td>5.59 (1H, s)</td>
<td>147.7</td>
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<tr>
<td>5'</td>
<td>1.48 (3H, s)</td>
<td>21.2</td>
<td>147.7</td>
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</table>

Figure 2. The key HMBC (——), \(^1H\)–\(^1H\) COSY (——) correlations of 1.
looks like a mirror image of the experimental ECD of (−)-1 (Fig. 4A). When compared to the calculated ECD of (3R,4S) with the ECD of (+)-1 (Fig. 4B), the ECD curves matched each other well (Fig. 4B). Therefore, the real AC for (−)-1 should have (3S,4R). This conclusion agrees well with the prediction using matrix method and X-ray experiment.11

Bioassay

Compound 1 was evaluated for cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7, SW480 cells by MTS assay. These assays did not show the tumor growth inhibitory activity in vitro. There was also no potent inhibiting the nitric oxide release. We also investigated if compound 1 has acetylcholinesterase inhibition activity.12 Our results showed that (−)-1 could inhibit the acetylcholinesterase activity by 18.7% (±0.26) at the concentration of 50 µM, and compound (+)-1 with the concentration of 50 µM could inhibit the activity of acetylcholinesterase by up to 32.4% (±2.08), compared with 43.6% (±2.12) inhibition rate of the positive control tacrine. The different stereogenic centers have a little effect on the activity of anti-AChE.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.03.031.

References and notes

8. Penicilliumine (1), C20H26N6O5, colorless block; |ɛ|2866 = 30.0 (c 0.0012, acetonitrile). (−)-penicilliumine: |ɛ|280 = 28.0 (c 0.0008, acetonitrile), (+)-penicilliumine; UV (MeOH) 3max (logε) 202.2 (3.83), 222.4 (3.89), 253.4 (3.51); IR (KBr) vmax 3410, 2923, 1684, 1661, 1117 cm–1; 1H (600 MHz) and 13C NMR (150 MHz) data see Table 1; ESI/MS (positive) m/z = 419 [M+Na]+; HREIMS m/z = 396.1432 (calc for 396.1434, C20H26N6O5).
11. Crystal data for penicilliumine: 2 (C20H26N6O5)2CO2, M = 836.81, monoclinic, a = 16.6711(3) Å, b = 13.7624(2) Å, c = 18.3999(3) Å, β = 90.00°, V = 108.8250(10) Å3, Z = 4, µ(CuKα) = 0.876 mm–1, 16367 reflections measured, 3534 independent reflections (Rint = 0.0441). The final R1 values were 0.066 (I > 2σ(I)). The final wR2(F) values were 0.2023 (I > 2σ(F)). The final R1 values were 0.0702 (all data). The final wR2(F) values were 0.2067 (all data). The goodness of fit on F2 was 1.054.
12. Acetylcholinesterase (AChE) inhibitory activity of the compounds isolated was assayed by the spectrophotometric method developed by Ellman et al.13 with slight modification. 5-Acetyltiothiocholine iodide; 5-butyryltiothiocholine iodide, 5,5′-dithio-bis-(2-nitrobenzoic) acid (DTNB, Ellman’s reagent), and acetylcholinesterase derived from human erythrocytes were purchased from Sigma Chemical. Compounds were dissolved in DMSO. The reaction mixture (totally 200 µL) containing phosphate buffer (pH 8.0), test compound (50 µM), and acetyl cholinesterase (0.02 U/mL) was incubated for 20 min (37 °C). Then, the reaction was initiated by the addition of 40 µL of solution containing DTNB (0.625 mM) and acetylthiocholine iodide (0.625 mM) for AChE inhibitory activity assay, respectively. The hydrolysis of acetylthiocholine was monitored at 405 nm every 30 s for 1 h. Tacrine was used as positive control with a final percentage inhibition was calculated as follows: % inhibition = (E - S)/E × 100 (E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound).