Coumarins, dihydroisocoumarins, a dibenzo-α-pyrone, a meroterpenoid, and a merodrimane from *Talaromyces amestolkiae*

By: Tamam El-Elimat, Mario Figueroa, Huzefa A. Raja, Soraya Alnabulsi, and Nicholas H. Oberlies


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Abstract:

Chemical investigation of an organic extract of a fungus isolated from submerged wood collected from fresh water (strain G173), identified as a *Talaromyces amestolkiae* (Eurotiales; Trichocomaceae), led to the isolation of three coumarins, three dihydroisocoumarins, a dibenzo-α-pyrone, a meroterpenoid, and a merodrimane. Three of the isolated compounds, namely 7-chloropestalasin A (3), 4-hydroxyaspergillumarin (6), and *ent*-thailandolide B (9) were new. The structures were elucidated using a combination of spectroscopic and spectrometric techniques. The absolute configurations of 2, 3, 5, and 6 were established via a modified Mosher’s ester method, whereas for 9 a combination of TDDFT ECD and ORD calculations were employed. Compounds 1–9 were evaluated for antimicrobial activity against a group of bacteria and fungi.

Keywords: Freshwater Fungi | Coumarins | Dihydroisocoumarins | Dibenzo-α-pyrones | Meroterpenoids | Merodrimanes

Article:
As part of an ongoing project to uncover new chemistry from nature [1], [2], [3], [4], [5], our group has been investigating freshwater fungi [6], [7], [8], [9], [10], [11]. Lignicolous freshwater fungi represent a viable resource for discovering new secondary metabolites with a broad range of biological activities [12], [13], [14].

A fungal strain accessioned as G173 and identified as *Talaromyces* amestolkiae (Eurotiales; Trichocomaceae) was isolated from submerged wood in a small pond near Bur-Mil Park, Guilford County, North Carolina. From an ecological point of view, strain G173 is not a true indweller of freshwater but can be defined as an immigrant species [15], [16]. Fractionation of the organic extract of G173 using flash chromatography, followed by preparative RP-HPLC, resulted in the isolation of three coumarins (1–3), three dihydroisocoumarins (4–6), a dibenzo-α-pyrone (7), a meroterpenoid (8), and a merodrimane (9), with >97% purity according to UPLC-PDA (Fig. S1).

Compounds 1 (12.2 mg) and 2 (1.0 mg) were isolated as colorless amorphous solids with molecular formulas of C_{12}H_{12}O_{5} and C_{14}H_{16}O_{5}, respectively, as determined by HRESIMS. The NMR (Fig. S2) and HRMS data identified 1 as the known compound, 3-hydroxymethyl-6,8-dimethoxycoumarin (Fig. 1), which was previously isolated from the soil fungus *T. flavus* [17]. In addition, 2 was identified as pestalasin A (Fig. S3), a coumarin that was reported from the endophytic fungus *Pestalotiopsis* sp., which was isolated from the leaves of the Chinese mangrove *Rhizophora mucronata* [18]. The absolute configuration of 2 was not previously reported; therefore it was assigned via a modified Mosher’s ester method [19], establishing the configuration as 2′S (Figs. 2 and S4).

![Structures of compounds 1–9.](image-url)
Fig. 2. $\Delta \delta_H$ values [$\Delta \delta$ (in ppm) = $\delta_S$ − $\delta_R$] obtained for (S)- and (R)-MTPA esters 2a and 2b for pestalasin A (2), 3a and 3b for 7-chloro-pestalasin A (3), and 6a and 6b for 4-hydroxyaspergillumarin A (6), in pyridine-$d_5$.

Compound 3 (0.5 mg) was obtained as a white solid [20]. The molecular formula was determined as C₁₄H₁₅ClO₅ by HRESIMS and analysis of $^1$H, HMBC, and edited-HSQC NMR data (Table 1, Fig. 3, and Figs. S5–S7). The HRMS and NMR data indicated 3 as a chlorinated analogue of 2, which was supported by both the presence of the characteristic isotopic pattern of chlorine in the HRMS data of 3, and the replacement of the meta-coupled aromatic protons ($\delta_H$ 6.45 and 6.65 for H-5 and H-6, respectively, $J_{H-5/H-7} = 2.65$ Hz) in 2 (Fig. S3), by a singlet aromatic proton ($\delta_H$ 6.70 for H-5) in 3 (Fig. S5). Analyses of the 2D NMR data (Fig. 3) gave the structure of 3, which was ascribed the trivial name 7-chloropestalasin A. The absolute configuration of 3 was assigned via a modified Mosher’s ester method [19], establishing the configuration as $2'S$ (Figs. 2 and S8).

Table 1. $^1$H and $^{13}$C NMR data of 3 (400 MHz for $^1$H; 100 MHz for $^{13}$C, CDCl₃) and 6 (700 MHz for $^1$H; 175 MHz for $^{13}$C, CDCl₃).

<table>
<thead>
<tr>
<th>Position</th>
<th>$^1$H δc (ppm)</th>
<th>$^1$H $J$ (Hz)</th>
<th>$^{13}$C δc (ppm)</th>
<th>$^{13}$C $J$ (Hz)</th>
</tr>
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<tr>
<td>1</td>
<td>168.8</td>
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<td></td>
<td></td>
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<tr>
<td>2</td>
<td>161.4</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>3</td>
<td>128.4</td>
<td>7.95, s</td>
<td>83.3</td>
<td>4.43, ddd (8.6, 3.4, 2.9)</td>
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<td>67.4</td>
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</tr>
<tr>
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<td></td>
<td></td>
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</tr>
<tr>
<td>5</td>
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<td>116.1</td>
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<tr>
<td>6</td>
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<td>117.8</td>
<td>6.98, d (8.0)</td>
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<tr>
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<td>106.8</td>
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<td>9</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>5'</td>
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<td>2.16, s</td>
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<tr>
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<td>2.76, br. s.</td>
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<tr>
<td>8-OH</td>
<td></td>
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<td>10.91, s</td>
</tr>
</tbody>
</table>

*$^{13}$C NMR data for 3 were obtained from HMBC and edited-HSQC spectra.
Compounds 4 (10.5 mg; colorless oil) and 5 (2.0 mg; colorless crystal) were isolated with molecular formulas of C_{14}H_{16}O_{4} and C_{14}H_{18}O_{4}, respectively, as determined by HRESIMS. The NMR (Figs. S9 and S10) and HRMS data identified 4 and 5 as the known dihydroisocoumarins, aspergillumars A and B, respectively (Fig. 1), which were previously reported from the culture broth of a marine-derived fungus *Aspergillus* sp., isolated from the fresh leaf of the mangrove tree *Bruguiera gymnorrhiza* collected from the South China Sea [21]. The NMR data of 5 matched those reported by Li and co-workers, except for the chemical shift of the 5′-methyl group (δH 2.34, d, J = 6 Hz) [21], which was observed at δH 1.22, d, J = 6 Hz (Fig. S10). The absolute configuration of 5 at C-4′ was not determined by Li and co-workers [21]. Therefore, we attempted to assign the absolute configuration via a modified Mosher’s ester method [19]; however, these results indicated that 5 was a racemic mixture. Indeed, four products were observed, a major and a minor product from each reaction in a 3:1 ratio (Fig. S11).

Compound 6 (0.6 mg) was obtained as a white solid [22], with a molecular formula of C_{14}H_{16}O_{5} as determined by HRESIMS along with ^1H, ^13C, and edited-HSQC NMR data (Table 1, Figs. S12 and S13), establishing an index of hydrogen deficiency (IHD) of 7. The NMR data suggested 6 as a dihydroisocoumarin analogue of 4. A key difference was replacement of the allylic methylene moiety (δH/δC 2.93/34.1, m, for H2-4/C-4) in 4 by an oxymethine in 6 (δH/δC 4.78/67.4, dd, J = 8.6, 2.3 for H-4/C-4). These data, along with a 16 amu difference in the HRMS data between 4 and 6, indicated hydroxylation at the C-4 position in 6. The coupling constant (J_{H-4/H-3} = 8.6 Hz) implied a pseudoaxial/pseudoequatorial trans orientation in 6 (Table 1, Fig. S12). A NOESY correlation observed between 4-OH and H-3 indicated that these two protons were on the same face (Figs. 3 and S15). Analyses of the COSY and HMBC NMR data (Figs. 3 and S14), established the structure of 6, which was given the trivial name 4-hydroxyaspergillumarin A. The absolute configuration of 6 was assigned via a modified Mosher’s ester method [19] as 4S (Figs. 2 and S16).
Compounds 7 (5.8 mg) and 8 (6.3 mg) were isolated as colorless crystalline solids and identified using HRMS and NMR data as graphisolactone A (a dibenzo-α-pyrone) [23] and berkeleyacetal C (a meroterpenoid) [24] (Figs. S17 and S18), respectively. Graphisolactone A was first isolated from the lichen Graphis scripta var. pulverulenta [25], while berkeleyacetal C was isolated from extracts of a Penicillium sp. [24].

Compound 9 (2.9 mg) was obtained as a white solid [26], with a molecular formula of C_{27}H_{32}O_{8} as determined by HRESIMS and NMR data (Table S3 and Figs. 3 and S19–S22), establishing an IHD of 12. The HRMS and NMR data of 9, including the NOESY spectrum, were identical to that of thailandolide B, a merodrimane isolated from T. thailandiasis [27]. However, the specific rotation of 9 ([α]_{D}^{20} − 47, CHCl₃, c 0.05) was found to be opposite to that of thailandolide B ([α]_{D}^{24} + 134, CHCl₃, c 0.1), suggesting that 9 could be an enantiomer of thailandolide B [27]. Thus, the absolute configuration of 9 was determined using electronic circular dichroism (ECD) and optical rotatory dispersion (ORD) spectroscopy combined with time-dependent density functional theory (TDDFT) and quantum chemical calculations. The calculated TDDFT-ECD spectrum of 9 matched the measured data, displaying two positive (~230 and ~310 nm) and two negative (~270 and ~350 nm) Cotton effects, respectively (Fig. 4). The calculated spectra for thailandolide B was, as expected, opposite to 9 (Fig. 4). Unfortunately, no experimental data were published for thailandolide B for comparison purposes. However, the calculated ORD value for 9 ([α]_{D}^{20} − 88.5 in CHCl₃) agreed with the experimental data. Thus, the absolute configuration of 9 was established as 5S,7R,8S,9S,10R,18S,19S and given the trivial name ent-thailandolide B.

Fig. 4. Comparison of experimental and calculated ECD spectra of 9 and thailandolide B in MeOH.

Compounds 1–9 were tested for antimicrobial activity against a group of bacteria and fungi [28] and found to be inactive.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2021.153067.

References


[20] 7-Chloropestalasin A (3): white solid; $[\alpha]_D^{20} = +23$ ($c = 0.05$, Chloroform); $^1$H NMR (CDCl$_3$, 400 MHz); see Table S1 and Fig. S5-S7; HRESIMS $m/z$ 299.0670 [M+H]$^+$ (calcd for C$_{14}$H$_{16}$ClO$_5$ 299.0681); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 295 (3.02), 258 (2.97), 214 (3.14) nm.


[22] 4-Hydroxyaspergilluarin A (6): white solid; $[\alpha]_D^{20} = +1$ ($c = 0.04$, Chloroform); $^1$H NMR (CDCl$_3$, 700 MHz) and $^{13}$C NMR (CDCl$_3$, 175 MHz); see Table S2 and Fig. S12-S15; HRESIMS $m/z$ 265.1069 [M+H]$^+$ (calcd for C$_{14}$H$_{17}$O$_5$ 265.1071); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 315 (3.16), 245 (3.18), 220 (3.10) nm.


[26] ent-thailandolide B (9): white solid; $[\alpha]_D^{20} = -47$ ($c = 0.05$, Chloroform); $^1$H NMR (CDCl$_3$, 500 MHz) and $^{13}$C NMR (CDCl$_3$, 125 MHz); see Table S3 and Fig. S19-S22; HRESIMS $m/z$ 485.2153 [M+H]$^+$ (calcd for C$_{27}$H$_{33}$O$_8$ 485.2170); UV (MeOH) $l_{max}$ (log $\varepsilon$) 314 (3.42), 271 (3.57), 237 (3.61) nm.