

## Coumarins, dihydroisocoumarins, a dibenzo- $\alpha$ -pyrone, a meroterpenoid, and a merodrimane from *Talaromyces amestolkiae*

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Tamam El-Elimat, Mario Figueroa, Huzefa A. Raja, Soraya Alnabulsi, Nicholas H. Oberlies. Coumarins, dihydroisocoumarins, a dibenzo- $\alpha$ -pyrone, a meroterpenoid, and a merodrimane from *Talaromyces amestolkiae*. *Tetrahedron Letters*. 2021, Article number 153067. DOI: 10.1016/j.tetlet.2021.153067

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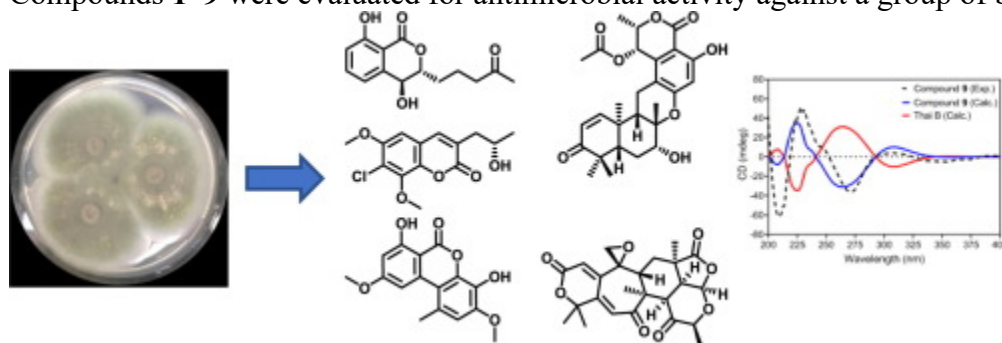


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### Abstract:

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- $\alpha$ -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.



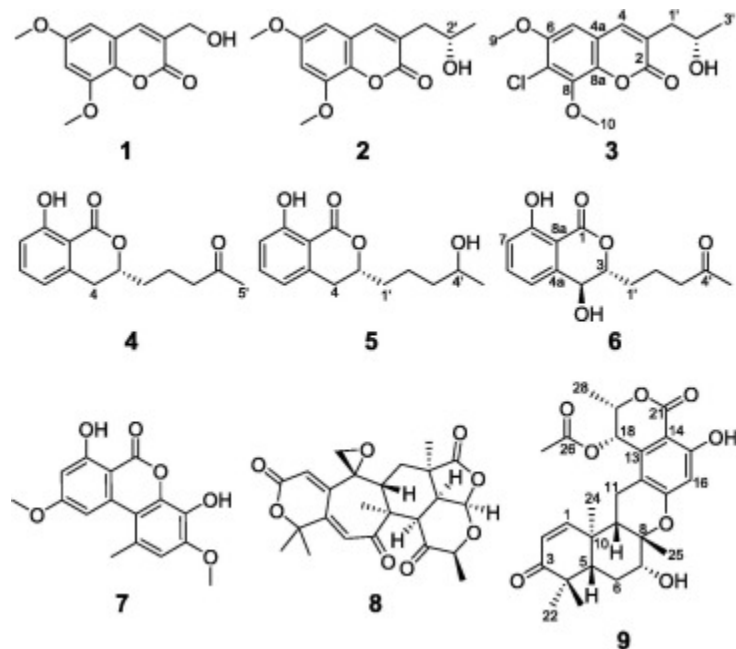
**Keywords:** Freshwater Fungi | Coumarins | Dihydroisocoumarins | Dibenzo- $\alpha$ -pyrones | Meroterpenoids | Merodrimanes

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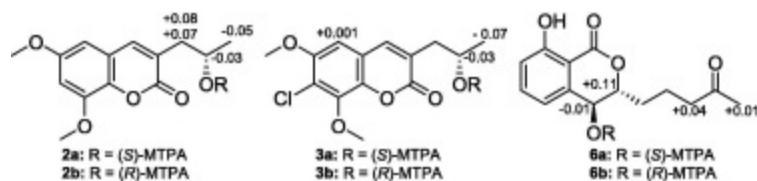
As part of an ongoing project to uncover new chemistry from nature [1], [2], [3], [4], [5], our group has been investigating freshwater fungi [6], [7], [8], [9], [10], [11]. Lignicolous freshwater fungi represent a viable resource for discovering new secondary metabolites with a broad range of biological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



**Fig. 1.** Structures of compounds **1–9**.



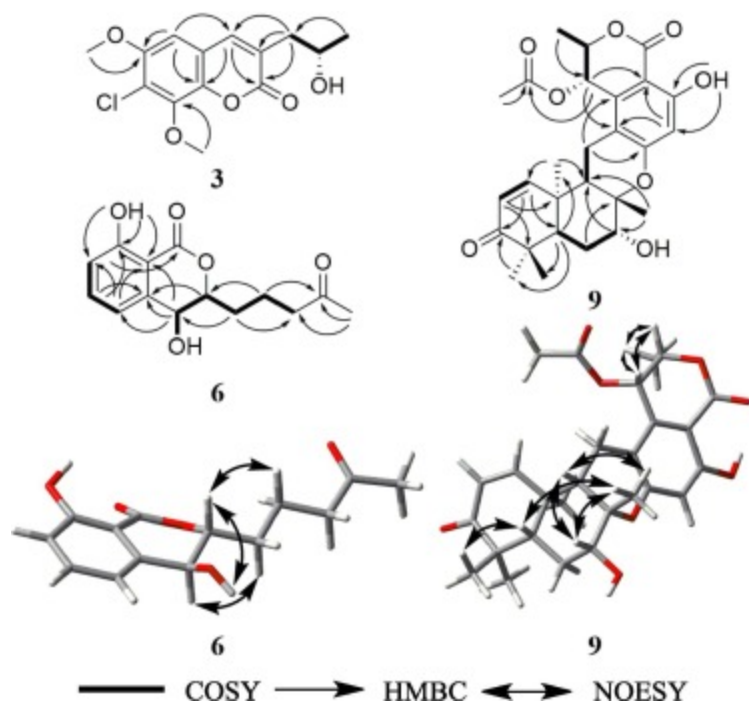
**Fig. 2.**  $\Delta\delta_{\text{H}}$  values [ $\Delta\delta$  (in ppm) =  $\delta_{\text{S}} - \delta_{\text{R}}$ ] obtained for (S)- and (R)-MTPA esters **2a** and **2b** for pestalasin A (**2**), **3a** and **3b** for 7-chloro-pestalasin A (**3**), and **6a** and **6b** for 4-hydroxyaspergillum A (**6**), in pyridine-*d*<sub>5</sub>.

Compound **3** (0.5 mg) was obtained as a white solid [20]. The molecular formula was determined as C<sub>14</sub>H<sub>15</sub>ClO<sub>5</sub> by HRESIMS and analysis of <sup>1</sup>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 ( $\delta_{\text{H}}$  6.45 and 6.65 for H-5 and H-6, respectively,  $J_{\text{H-5/H-6}} = 2.65$  Hz) in **2** (Fig. S3), by a singlet aromatic proton ( $\delta_{\text{H}}$  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-chloro-pestalasin 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.** <sup>1</sup>H and <sup>13</sup>C NMR data of **3** (400 MHz for <sup>1</sup>H; 100 MHz for <sup>13</sup>C, CDCl<sub>3</sub>) and **6** (700 MHz for <sup>1</sup>H; 175 MHz for <sup>13</sup>C, CDCl<sub>3</sub>).

Position	<b>3</b>		<b>6</b>	
	$\delta_{\text{C}}^*$	$\delta_{\text{H}}$ mult ( <i>J</i> in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ mult ( <i>J</i> in Hz)
1			168.8	
2	161.4			
3	128.4		83.3	4.43, ddd (8.6, 3.4, 2.9)
4	137.6	7.95, s	67.4	4.78, dd (8.6, 2.3)
4a	109.6		141.9	
5	100.0	6.70, s	116.1	7.07, d (7.5)
6	151.8		137.1	7.53, dd (8.0, 7.5)
7	118.1		117.8	6.98, d (8.0)
8	146.1		162.2	
8a	137.9		106.8	
9	57.4	3.92, s		
10	56.9	3.95, s		
1'	41.1	2.64, dd (13.7, 8.2) 2.83, dd (13.7, 3.7)	30.7	1.76, m 1.92, m
2'	66.7	4.16, m	18.4	1.75, m 1.90, m
3'	23.7	1.28, d (6.4)	42.9	2.56, ddd (9.2, 6.3, 2.9)
4'			209.2	
5'			30.3	2.16, s
4-OH				2.76, br. s.
8-OH				10.91, s

\*<sup>13</sup>C NMR data for **3** were obtained from HMBC and edited-HSQC spectra.



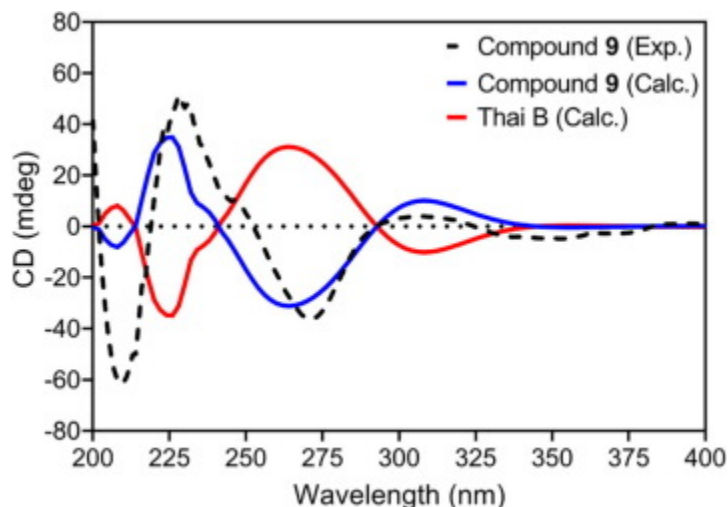
**Fig. 3.** Key HMBC, COSY, and NOESY correlations of **3**, **6** and **9**.

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, aspergillumarins 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 ( $\delta_H$  2.34, d,  $J = 6$  Hz) [21], which was observed at  $\delta_H$  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  $^1H$ ,  $^{13}C$ , 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 ( $\delta_H/\delta_C$  2.93/34.1, m, for  $H_2-4/C-4$ ) in **4** by an oxymethine in **6** ( $\delta_H/\delta_C$  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<sup>19</sup> as 4*S* (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 graphislactone A (a dibenzo- $\alpha$ -pyrone) [23] and berkeleyacetal C (a meroterpenoid) [24] (Figs. S17 and S18), respectively. Graphislactone 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** ( $[\alpha]_D^{20} - 47$ ,  $CHCl_3$ ,  $c$  0.05) was found to be opposite to that of thailandolide B ( $[\alpha]_D^{24} + 134$ ,  $CHCl_3$ ,  $c$  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 ( $\sim 230$  and  $\sim 310$  nm) and two negative ( $\sim 270$  and  $\sim 350$  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** ( $[\alpha]_D^{20} - 88.5$  in  $CHCl_3$ ) 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

This research was supported in part by the National Institutes of Health/National Cancer Institute *via* P01 CA125066. TE acknowledges the partial financial support by Deanship of Research, Jordan University of Science and Technology, Irbid, Jordan (Grant No. 38/2018). MF acknowledges partial financial support *via* grants from UNAM-DGAPA IN222220 and FQ-PAIP 5000-9145. High-resolution mass spectrometry data were acquired at the Triad Mass Spectrometry Laboratory at the University of North Carolina at Greensboro. This work was performed in part at the Joint School of Nanoscience and Nanoengineering, a member of the Southeastern Nanotechnology Infrastructure Corridor (SENIC) and National Nanotechnology Coordinated Infrastructure (NNCI), which is supported by the National Science Foundation (Grant ECCS-1542174).

## Appendix A. Supplementary data

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

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