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

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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

As part of an ongoing project to uncover new chemistry from nature,[1–5] our group has been investigating freshwater fungi.[6–11] Lignicolous freshwater fungi represent a viable resource for discovering new secondary metabolites with a broad range of biological activities.[12–14]

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-

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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 *Talaromyces 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* (Fig. 2 and S4).

Compound **3** (0.5 mg) was obtained as a white solid.[20] The molecular formula was determined as $C_{14}H_{15}ClO_5$ by HRESIMS and analysis of ^{1}H, HMBC, and edited-HSQC NMR data (Table 1 and Fig. 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 (δ_H 6.45 and 6.65 for H-5 and H-6, respectively, $J_{H\text{-}5/H\text{-}7}$ = 2.65 Hz) in **2** (Fig. S3), by a singlet aromatic proton (δ_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* (Fig. 2 and S8).

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 (Fig. 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 (δ_H 2.34, d, J = 6 Hz),[21] which was observed at δ_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 ^{1}H, ^{13}C, and edited-HSQC NMR data (Table 1, Fig. S12 and S13), establishing an index of hydrogen deficiency of 7. The NMR data suggested **6** as a dihydroisocoumarin analogue of **4**. A key difference was replacement of the allylic methylene moiety (δ_H/δ_C 2.93/34.1, m, for H_2-4/C-4) in **4** by an oxymethine in **6** (δ_H/δ_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 ($J_{H\text{-}4/H\text{-}3}$ = 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 (Fig. 3 and S15). Analyses of the COSY and HMBC NMR data (Fig. 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 4*S* (Fig. 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-α-pyrone)[23] and berkeleyacetal C (a meroterpenoid)[24] (Fig. 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 Fig. 3 and S19–S22), establishing an index of hydrogen deficiency of 12. The HRMS and NMR data of **9**, including the NOESY spectrum, were identical to that of thailandolide B, a merodrimane isolated from *Talaromyces 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 (~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** ($[\alpha]_D^{20}$ −88.5 in $CHCl_3$) agreed with the experimental data. Thus, the absolute configuration of **9** was established as 5*S*,7*R*,8*S*,9*S*,10*R*,18*S*,19*S* and given the trivial name *ent*-thailandolide B.

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

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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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) λ_{max} (log ε) 295 (3.02), 258 (2.97), 214 (3.14) nm.
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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 *e*) 314 (3.42), 271 (3.57), 237 (3.61) nm.

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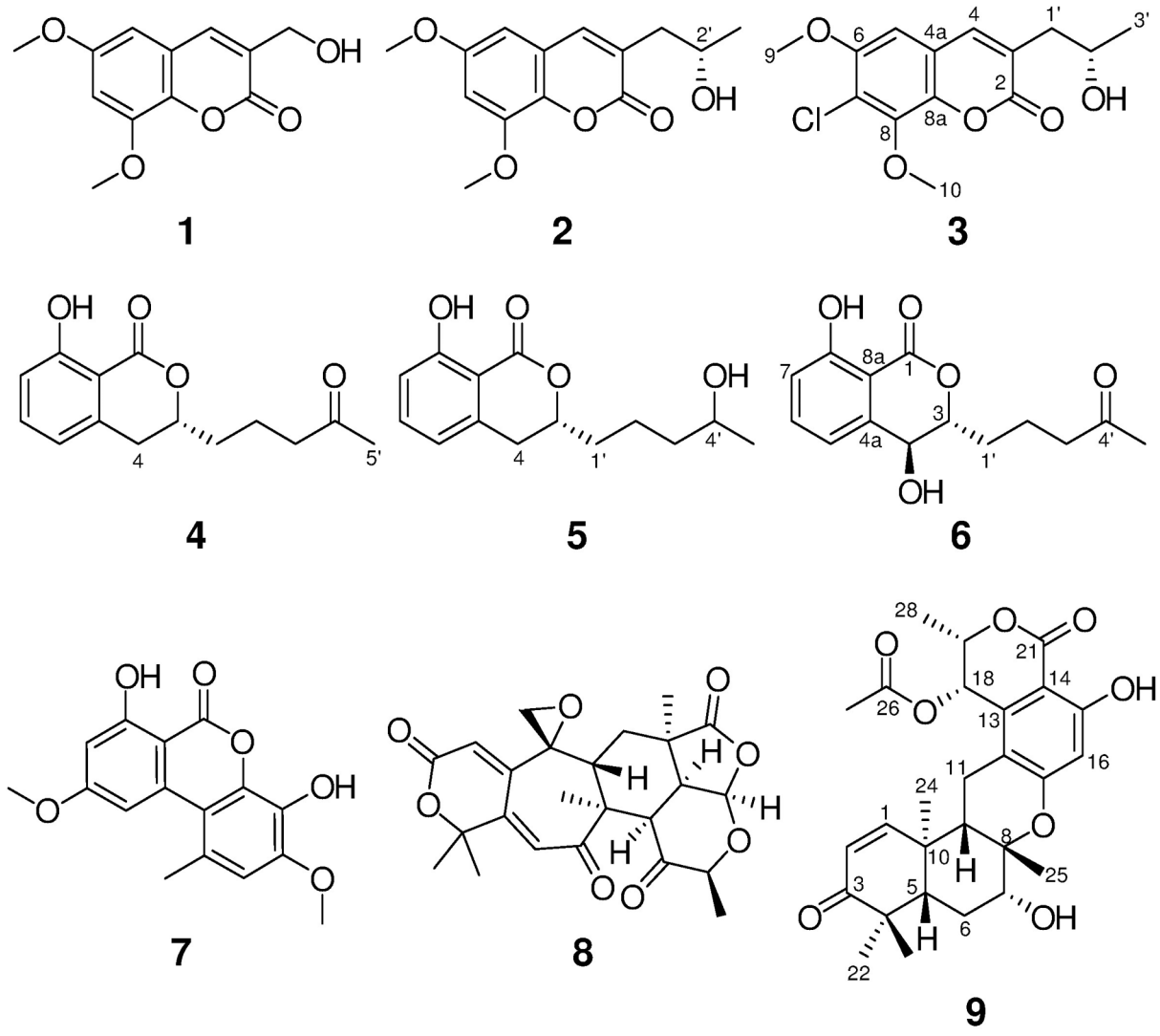

Fig. 1.
Structures of compounds **1**-**9**.

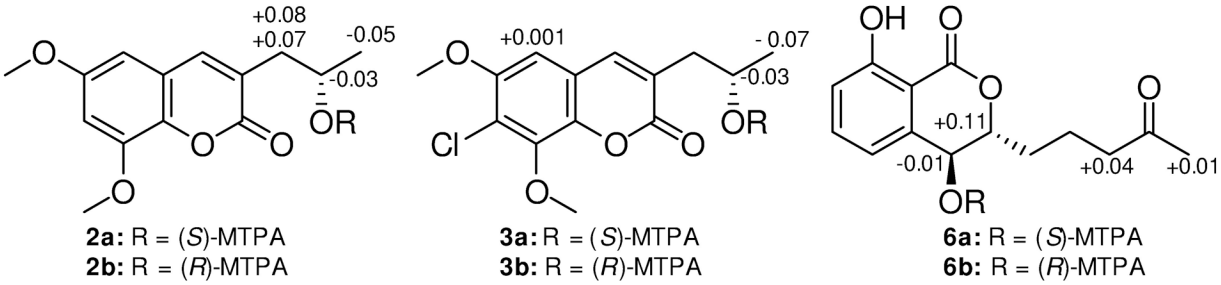

Fig. 2.

$\Delta\delta_H$ 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-hydroxy-aspergillumarin A (**6**), in pyridine-d_5.

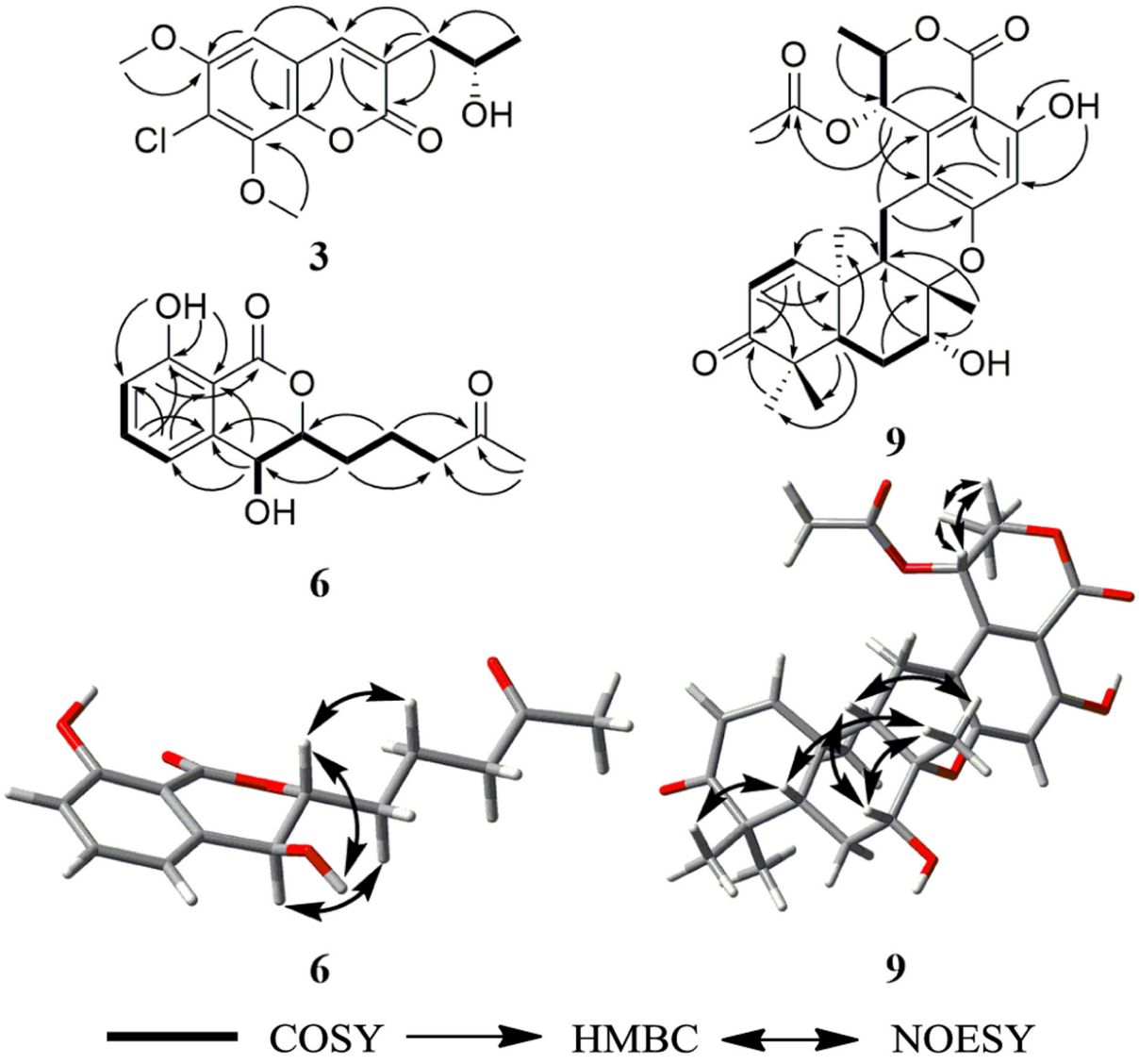

Fig. 3.
Key HMBC, COSY, and NOESY correlations of **3**, **6** and **9**.

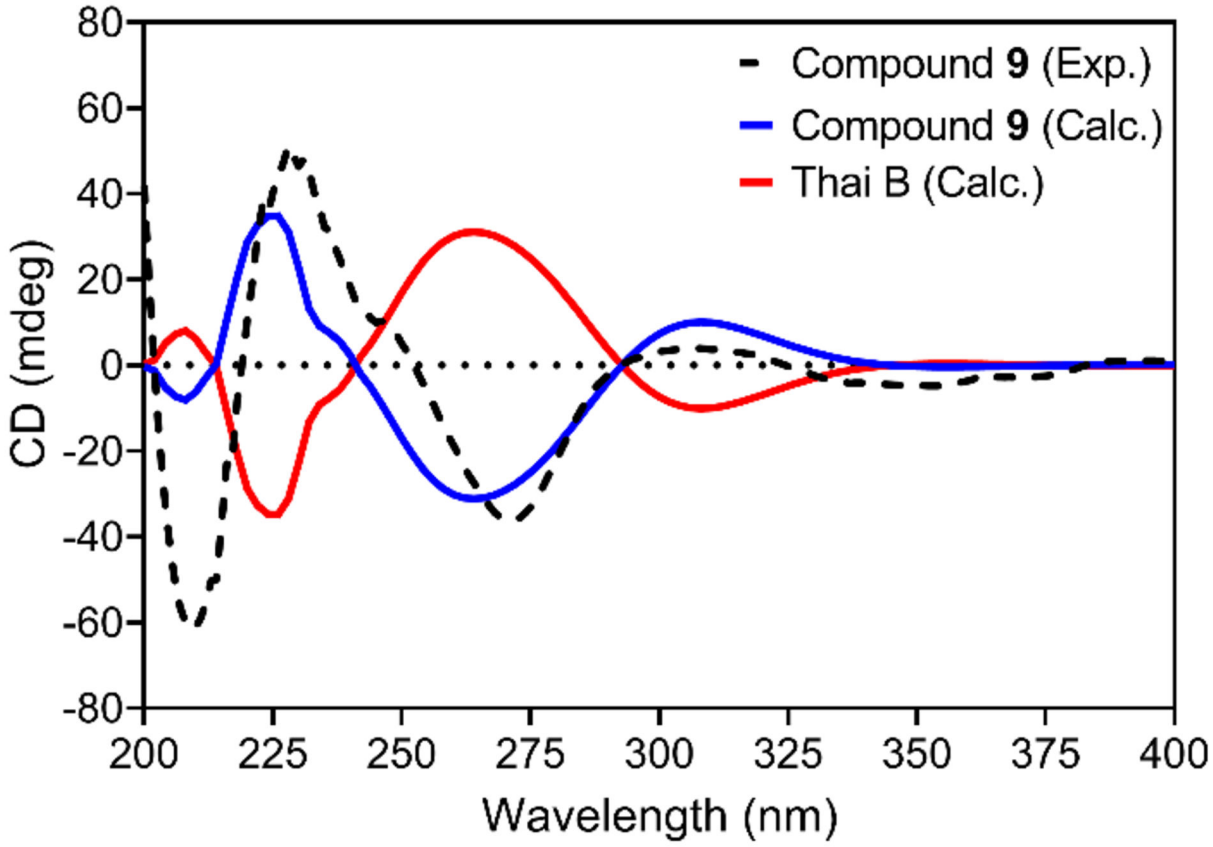

Fig. 4.
Comparison of experimental and calculated ECD spectra of **9** and thailandolide B in MeOH.

Table 1.

^{1}H and ^{13}C NMR data of **3** (400 MHz for ^{1}H; 100 MHz for ^{13}C, $CDCl_3$) and **6** (700 MHz for ^{1}H; 175 MHz for ^{13}C, $CDCl_3$)

Position	3		6	
	δ_C *	δ_H mult (*J* in Hz)	δ_C	δ_H mult (*J* 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)	30.7	1.76, m
		2.83, dd (13.7, 3.7)		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

*^{13}C NMR data for **3** were obtained from HMBC and edited-HSQC spectra.