

# Delitschiapyrone A, a Pyrone–Naphthalenone Adduct Bearing a New Pentacyclic Ring System from the Leaf-Associated Fungus *Delitschia* sp. FL1581

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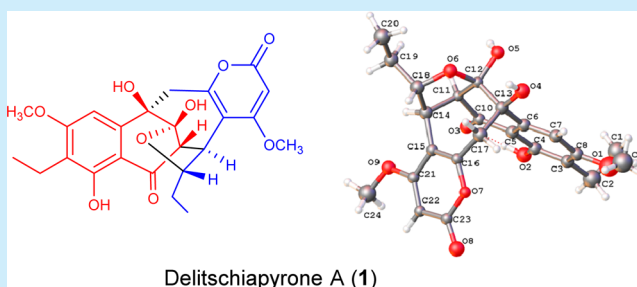
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## S Supporting Information

**ABSTRACT:** Delitschiapyrone A (**1**), an  $\alpha$ -pyrone–naphthalenone adduct with an unprecedented pentacyclic ring system, was isolated from a solid culture of the leaf-associated fungus *Delitschia* sp. FL1581. The structure of **1** was elucidated by spectroscopic analysis and X-ray crystallography, and its absolute configuration was defined by experimental and calculated ECD. Biosynthetically, the unique 6/6/5/7/6 pentacyclic core of **1** may be formed by an intermolecular Diels–Alder-type addition of the precursors derived from (1'R)-2',3'-dihydropyrenocine C (**2**) and 6-ethyl-2,7-dimethoxyjuglone (**3**) found to co-occur with **1** in this fungus.



Delitschiapyrone A (**1**)

Plants support a diverse array of microorganisms that profoundly influence plant health and productivity. Although mechanisms of interactions between microorganisms and their host plants are often not fully understood, many plant-associated fungi produce small-molecule natural products that represent a rich source of biologically active metabolites with wide-ranging applications as agrochemicals, antibiotics, immune-suppressants, antiparasitics, and anticancer agents.<sup>1</sup> As a part of our systematic search for new and/or bioactive small-molecule natural products from plant-associated fungi,<sup>2</sup> we investigated *Delitschia* sp. FL1581, isolated from the fallen leaves of *Serenoa repens* (saw palmetto) collected in south-central Florida. *Delitschia* is best known for occurring on decaying wood and fallen leaves.<sup>3</sup> To date, only a few secondary metabolites have been isolated from the fungi of this genus including *N*-hydroxyimides,<sup>4a</sup> isochromenone,<sup>4b</sup> naphthoquinones,<sup>4b,c</sup> and bis-naphthopyrones.<sup>4d</sup> Through investigation of a weakly cytotoxic EtOAc extract derived from a solid (potato dextrose agar, PDA) culture of the fungal strain, *Delitschia* sp. FL1581 was found to afford delitschiapyrone A (**1**) possessing an unprecedented pentacyclic ring system (Figure 1) and the known compounds (1'R)-2',3'-dihydropyrenocine C (**2**)<sup>5</sup> and 6-ethyl-2,7-dimethoxyjuglone (**3**)<sup>4b</sup> (Supporting Information). Herein we report the details of the structure elucidation of delitschiapyrone A (**1**) and its cytotoxic activity and propose a putative biosynthetic origin of delitschiapyrone A (**1**) from **2** and **3**.

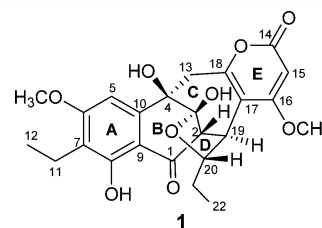


Figure 1. Structure of delitschiapyrone A (**1**).

The molecular formula of delitschiapyrone A (**1**)<sup>6</sup> was determined to be C<sub>24</sub>H<sub>26</sub>O<sub>9</sub> by a combination of its HRESIMS and <sup>13</sup>C NMR data, which required 12 degrees of unsaturation. The UV  $\lambda_{\text{max}}$  at 298 nm and IR absorption bands at 1694 and 1580 cm<sup>-1</sup> indicated the presence of an  $\alpha$ -pyrone moiety in **1**.<sup>7</sup> The <sup>1</sup>H and <sup>13</sup>C NMR spectra of **1** interpreted with the aid of DEPT, HSQC, and HMBC spectra revealed the presence of signals attributable to a chelated OH ( $\delta_{\text{H}}$  12.67), two nonchelated OH [ $\delta_{\text{H}}$  5.81 (br s) and 5.03 (br s)], two OCH<sub>3</sub> ( $\delta_{\text{H}}$  4.00 and 3.89), two CH<sub>3</sub>, three CH<sub>2</sub>, and five CH including one aromatic [ $\delta_{\text{H}}$  7.11 (s);  $\delta_{\text{C}}$  101.3] and one olefinic [ $\delta_{\text{H}}$  5.38 (s);  $\delta_{\text{C}}$  88.6], 11 quaternary carbons of which eight are aromatic/olefinic ( $\delta_{\text{C}}$  169.4, 165.5, 161.8, 159.5, 148.6, 118.6, 113.3, and 111.6), one acetal ( $\delta_{\text{C}}$  108.4), one

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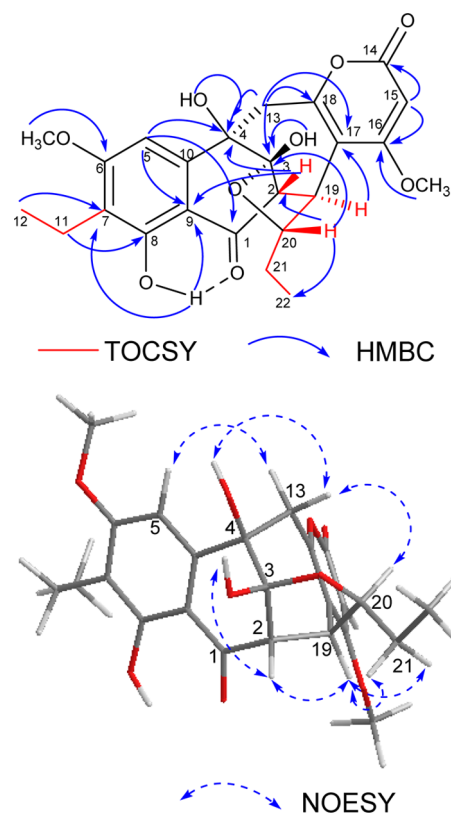
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oxymethine ( $\delta_{\text{C}}$  74.9), and two carbonyl carbons including those of the  $\alpha$ -pyrone ( $\delta_{\text{C}}$  162.8) and ketone ( $\delta_{\text{C}}$  200.0) moieties. These data accounted for all the NMR resonances of **1** (Table 1) and seven of the 12 unsaturation units, suggesting that **1** was pentacyclic and contained a trisubstituted  $\alpha$ -pyrone ring and a penta-substituted benzene ring (Figure 1).

**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data for **1** Recorded in Acetone- $d_6$  at 400 and 100 MHz, Respectively

no.	$\delta_{\text{H}}^{\text{a}}$ , mult (J, Hz)	$\delta_{\text{C}}^{\text{b}}$ , mult	HMBC
1		200.0 C	
2	3.52, d (8.8)	56.7 CH	1, 3, 4, 9, 17, 19
3		108.4 C	
4		74.9 C	
5	7.11, s	101.3 CH	1, 4, 6, 9, 10
6		165.5 C	
7		118.6 C	
8		161.8 C	
9		111.6 C	
10		148.6 C	
11	2.56, q (7.6)	16.1 CH <sub>2</sub>	6, 7, 8, 12
12	1.00, t (7.6)	13.5 CH <sub>3</sub>	7, 11
13	3.70, d (15.6)	46.7 CH <sub>2</sub>	3, 4, 10, 17, 18
	2.66, d (15.6)		3, 4, 10, 17, 18
14		162.8 C	
15	5.38, s	88.6 CH	14, 16, 17
16		169.4 C	15, 16
17		113.3 C	
18		159.5 C	
19	3.96, dd (8.8, 0.8)	39.0 CH	2, 3, 16, 17, 18, 20, 21
20	4.10, br t (6.8)	86.8 CH	2, 3, 17, 19, 22
21	1.71, dq (7.6, 7.2)	31.6 CH <sub>2</sub>	2, 19, 22
	1.78, dq (7.6, 7.2)		2, 19, 22
22	0.99, t (7.6)	10.5 CH <sub>3</sub>	20, 21
OMe-6	4.00, s	56.5 CH <sub>3</sub>	6
OMe-16	3.89, s	57.4 CH <sub>3</sub>	16
OH-3	5.81, br s		2, 3, 4
OH-4	5.03, br s		3, 4, 10, 13
OH-8	12.67, s		7, 8, 9

The TOCSY spectrum of **1** revealed the presence of spin systems due to  $-\text{CH}_2\text{CH}_3$  and  $-\text{CHCHCHCH}_2\text{CH}_3$  fragments (Figure 2). The HMBC correlations of  $\text{H}_3$ -12/C-7,  $\text{H}_2$ -11/C-6,  $\text{H}_2$ -11/C-8,  $\text{H}$ -5/C-9, OH ( $\delta_{\text{H}}$  12.67)/C-9, and OCH<sub>3</sub> ( $\delta_{\text{H}}$  4.00)/C-6, suggested that **1** contained an aromatic ring bearing H, OCH<sub>3</sub>, CH<sub>2</sub>CH<sub>3</sub>, and OH substituents at C-5, C-6, C-7, and C-8, respectively (Figure 2). Additionally, the HMBC cross-peaks of H-2/C-3, H-2/C-4, H-2/C-1 ( $\delta_{\text{C}}$  200.0), and H-5/C-4, as well as a weak but distinctive four-bond correlation from H-5 to C-1 allowed elaboration of the previously identified aromatic moiety in **1** to a naphthalen-1(4*H*)-one system (rings A and B; Figure 1).<sup>8</sup> The HMBC correlations of H-2 to C-4 and C-9 and H-20 to C-2 and C-22 suggested that the terminal CH of the fragment  $-\text{CHCHCHCH}_2\text{CH}_3$  was part of the naphthalen-1(4*H*)-one moiety (Figure 2). In the HMBC spectrum, correlations from the singlet olefinic signal at  $\delta_{\text{H}}$  5.38 (H-15) to the  $\alpha$ -pyrone carbonyl carbon C-14 ( $\delta_{\text{C}}$  162.8) and to C-16 ( $\delta_{\text{C}}$  169.4) and from OCH<sub>3</sub> ( $\delta_{\text{H}}$  3.89) to C-16 suggested that the  $\alpha$ -pyrone ring is 4,5,6-trisubstituted with an OCH<sub>3</sub> group at 4-position (C-16). The naphthalen-1(4*H*)-one and the  $\alpha$ -pyrone moieties in **1** were found to be linked through a CH<sub>2</sub> as protons of this group at  $\delta_{\text{H}}$  3.70 (d,  $J$  = 15.6

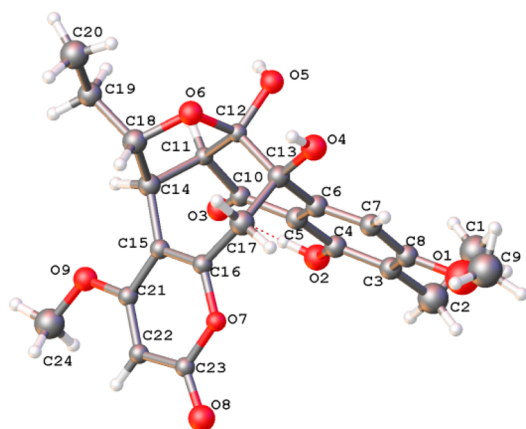


**Figure 2.** Selected key TOCSY, HMBC, and NOESY correlations of **1**.

Hz) and 2.66 (d,  $J$  = 15.6 Hz) showed HMBC correlations to C-3 and C-4 of the naphthalen-1(4*H*)-one moiety and C-17 and C-18 of the  $\alpha$ -pyrone moiety. The HMBC correlations of 20-H/C-2 and H-19/C-17 provided evidence for the second linkage between these two moieties generating a 7-membered C ring of **1** (Figure 1). The two nonchelated OH groups were located at C-3 and C-4 based on HMBC correlations observed from OH protons to their respective carbons. The above data accounted for the molecular formula of **1** except for one oxygen atom. On the basis of the chemical shift data for C-3 ( $\delta_{\text{C}}$  108.4) and C-20 ( $\delta_{\text{C}}$  86.8) and HMBC cross-peaks of H-20/C-3, this oxygen atom was determined to be linked to C-3 and C-20 generating the tetrahydrofuran ring D of **1**. Thus, the planar pentacyclic structure of **1** was completely defined as shown in Figure 1.

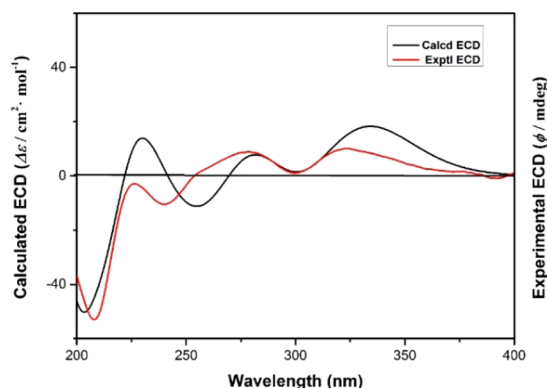
The relative configuration of delitschiapyrone A (**1**) was deduced by the analysis of its NOESY data. The NOESY correlations of H-2/3-OH, H-2/H-19, and H-19/H<sub>2</sub>-21 revealed these to be on the same side of the furan ring, while NOESY correlations of 4-OH/H-13a ( $\delta_{\text{H}}$  3.70), H-5/H-13b, and H-13a/H-20 placed the protons of 4-OH, H-13a, and H-20 on the same face of the seven-membered carbocyclic ring C.

Finally, the proposed structure of delitschiapyrone A (**1**) was confirmed by single-crystal X-ray diffraction using Mo  $K\alpha$  radiation. The perspective ORTEP plot is shown in Figure 3. Although the molecular structure was reliably determined, it was not possible to obtain the absolute configuration of **1** because its crystals were found to be twinned and poorly diffracting. In order to determine the absolute configuration, the theoretical calculations of the ECD spectra of **1** based on TD-SCF methods using GAUSSIAN-09 were adopted.<sup>9</sup> The initial structure of **1** obtained based on its crystal structure was used. The calculation was on a level of B3LYP/6-311+G(d,2p)



**Figure 3.** X-ray structure of **1**. (Note: A different numbering system is used for the structural data.)

using the TD-SCF method. Comparison of the experimental CD spectra with those calculated has previously been used to determine the absolute configurations of a variety of natural products.<sup>10</sup> As depicted in Figure 4, the predicted ECD for



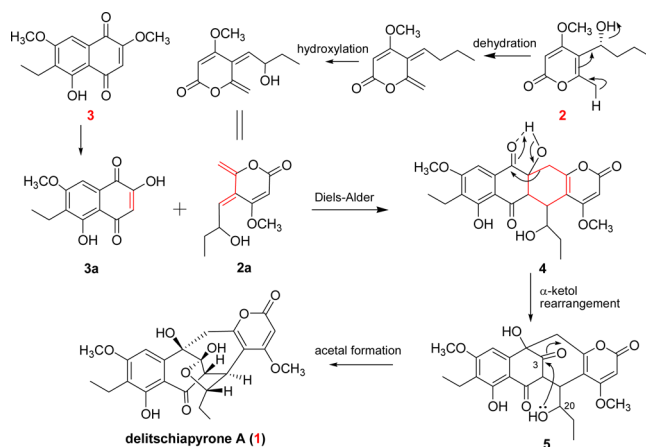
**Figure 4.** Calculated (black line) and experimental (red line) ECD of delitschiapyrone A (**1**).

2*R*,3*R*,4*R*,19*S*,20*S* configuration of **1** by theoretical calculation was found to be in agreement with its experimental ECD, allowing the assignment of the absolute configuration of delitschiapyrone A (**1**) as 2*R*,3*R*,4*R*,19*S*,20*S*.

To the best of our knowledge, delitschiapyrone A is the first example of a natural product containing a 6/6/7/5/6-fused ring system represented in **1**. Its structure determined from spectroscopic analysis and X-ray data provided evidence for the presence of this unprecedented pentacyclic ring system in which a naphthalenone and an  $\alpha$ -pyrone moiety were linked together by a seven-membered carbocyclic ring and a tetrahydrofuran ring leading to a stable folded conformation. Co-occurrence of **1** together with the  $\alpha$ -pyrone **2** and naphthoquinone **3** prompted us to postulate a biosynthetic pathway for **1** involving a Diels–Alder addition<sup>11</sup> followed by an  $\alpha$ -ketol-type rearrangement,<sup>12</sup> both of which have previously been proposed for the biosynthesis of a variety of natural products. Thus, the Diels–Alder reaction of the diene **2a** derived from **2** and the *O*-demethyl analogue of **3**, 6-ethyl-2,5-dihydroxy-7-methoxy-1,4-naphthoquinone (**3a**),<sup>13</sup> would lead to the key 6/6/6/6-fused tetracyclic intermediate **4**, which would then undergo an  $\alpha$ -ketol-type rearrangement<sup>12</sup> providing the 6/6/7/6 tetracyclic intermediate **5**. Subsequently, **5** would

undergo a cyclization reaction between 20-OH and the C-3 carbonyl group leading to an acetal and generating the tetrahydrofuran ring of **1** (Scheme 1).

#### Scheme 1. Proposed Biosynthesis of Delitschiapyrone A (**1**)



The <sup>1</sup>H and <sup>13</sup>C NMR data of **2** were identical with those reported for 2',3'-dihydropyrenocine C [5-(1'-hydroxybutyl)-4-methoxy-6-methyl-2-pyrone] obtained as a racemic mixture by the catalytic reduction of pyrenocine C, a metabolite of *Pyrenochaeta terrestris*.<sup>5</sup> Comparison of the [ $\alpha$ ]<sub>D</sub> of **2** (+15.7) with that of its analogue, macommelin-8-ol [5-(1'*S*-hydroxyethyl)-4-methoxy-6-methyl-2-pyrone] ([ $\alpha$ ]<sub>D</sub> −32.6),<sup>14</sup> identified **2** as (1'*R*)-2',3'-dihydropyrenocine C. The structure of compound **3** was determined as 6-ethyl-2,7-dimethoxyjuglone, which has previously been encountered in a *Delitschia* species.<sup>4b</sup>

Compounds **1**–**3** were evaluated for cytotoxicity against human tumor cell lines MCF-7, H460, HepG2, and U2OS. Compound **1** showed cytotoxic activity to all the cell lines with IC<sub>50</sub> values of 35.5, 12.9, 12.3, and 20.4  $\mu$ M, respectively, while **2** and **3** exhibited weaker activity than **1** (Table S1, Supporting Information).

## ■ ASSOCIATED CONTENT

### Supporting Information

General methods and details of isolation of metabolites, 1D and 2D NMR spectra, cytotoxic data for **1**–**3**, and crystallographic data file (CIF) for **1**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

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### Notes

The authors declare no competing financial interest.

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