Organic LETTERS

Letter

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

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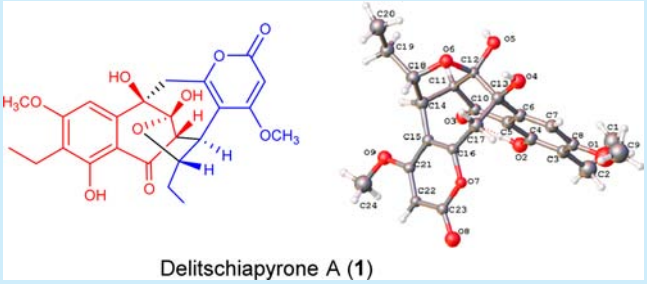
Supporting Information

ABSTRACT: Delitschiapyrone A (**1**), an α-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.



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.[1] As a part of our systematic search for new and/or bioactive small-molecule natural products from plant-associated fungi,[2] 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.[3] To date, only a few secondary metabolites have been isolated from the fungi of this genus including *N*-hydroxyimides,[4a] isochromenone,[4b] naphthoquinones,[4b,c] and bis-naphthopyrones.[4d] 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**)[5] and 6-ethyl-2,7-dimethoxyjuglone (**3**)[4b] (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**.

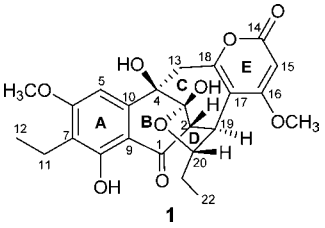


Figure 1. Structure of delitschiapyrone A (**1**).

The molecular formula of delitschiapyrone A (**1**)[6] was determined to be $C_{24}H_{26}O_9$ by a combination of its HRESIMS and ^{13}C NMR data, which required 12 degrees of unsaturation. The UV λ_{max} at 298 nm and IR absorption bands at 1694 and 1580 cm^{-1} indicated the presence of an α-pyrone moiety in **1**.[7] The ^1H and ^{13}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 (δ_H 12.67), two nonchelated OH [δ_H 5.81 (br s) and 5.03 (br s)], two OCH_3 (δ_H 4.00 and 3.89), two CH_3, three CH_2, and five CH including one aromatic [δ_H 7.11 (s); δ_C 101.3] and one olefinic [δ_H 5.38 (s); δ_C 88.6], 11 quarternary carbons of which eight are aromatic/olefinic (δ_C 169.4, 165.5, 161.8, 159.5, 148.6, 118.6, 113.3, and 111.6), one acetal (δ_C 108.4), one

Received: October 9, 2014
Published: November 3, 2014

oxymethine (δ_C 74.9), and two carbonyl carbons including those of the α-pyrone (δ_C 162.8) and ketone (δ_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 α-pyrone ring and a penta-substituted benzene ring (Figure 1).

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

no.	δ_H[a], mult (J, Hz)	δ_C[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_2	6, 7, 8, 12
12	1.00, t (7.6)	13.5 CH_3	7, 11
13	3.70, d (15.6)	46.7 CH_2	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_2	2, 19, 22
	1.78, dq, (7.6, 7.2)		2, 19, 22
22	0.99, t (7.6)	10.5 CH_3	20, 21
OMe-6	4.00, s	56.5 CH_3	6
OMe-16	3.89, s	57.4 CH_3	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 $-CH_2CH_3$ and $-CHCHCHCH_2CH_3$ fragments (Figure 2). The HMBC correlations of H_3-12/C-7, H_2-11/C-6 H_2-11/C-8, H-5/C-9, OH (δ_H12.67)/C-9, and OCH_3 (δ_H 4.00)/C-6, suggested that **1** contained an aromatic ring bearing H, OCH_3, CH_2CH_3, 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 (δ_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).[8] 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 $-CHCHCHCH_2CH_3$ was part of the naphthalen-1(4*H*)-one moiety (Figure 2). In the HMBC spectrum, correlations from the singlet olefinic signal at δ_H 5.38 (H-15) to the α-pyrone carbonyl carbon C-14 (δ_C 162.8) and to C-16 (δ_C 169.4) and from OCH_3 (δ_H 3.89) to C-16 suggested that the α-pyrone ring is 4,5,6-trisubstituted with an OCH_3 group at 4-position (C-16). The naphthalen-1(4*H*)-one and the α-pyrone moieties in **1** were found to be linked through a CH_2 as protons of this group at δ_H 3.70 (d, J = 15.6 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 α-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 (δ_C 108.4) and C-20 (δ_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.

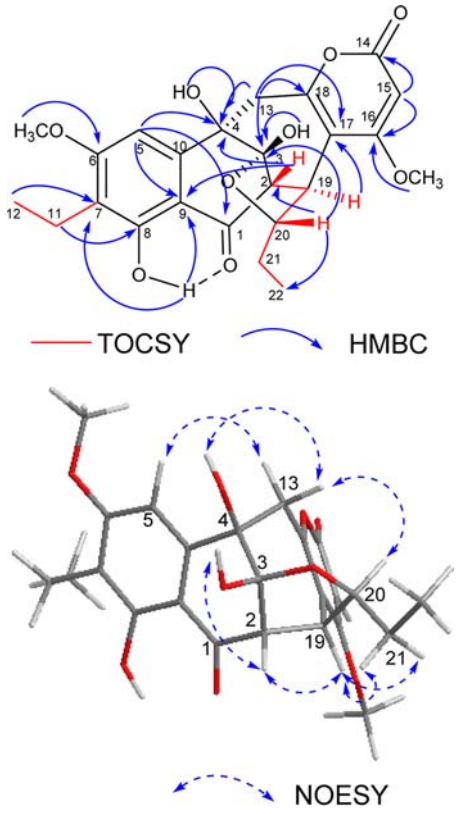


Figure 2. Selected key TOCSY, HMBC, and NOESY correlations of **1**.

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_2-21 revealed these to be on the same side of the furan ring, while NOESY correlations of 4-OH/H-13a (δ_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 Kα 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.[9] 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)

dx.doi.org/10.1021/ol502973c | *Org. Lett.* 2014, 16, 5944−5947

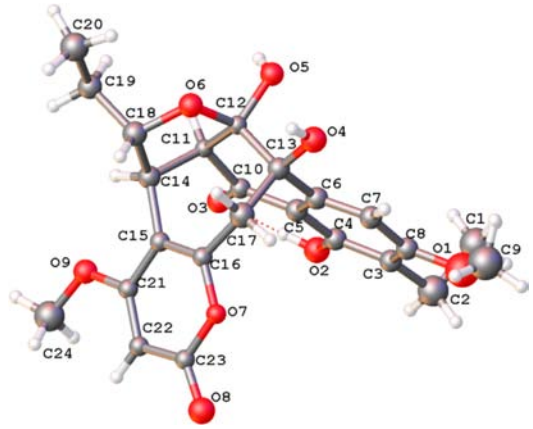


Figure 3. X-ray structure of **1**. (Note: A different numbering system is used for the structural data.)

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.[10] As depicted in Figure 4, the predicted ECD for

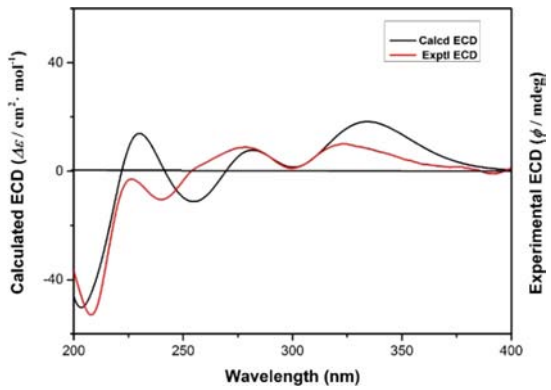
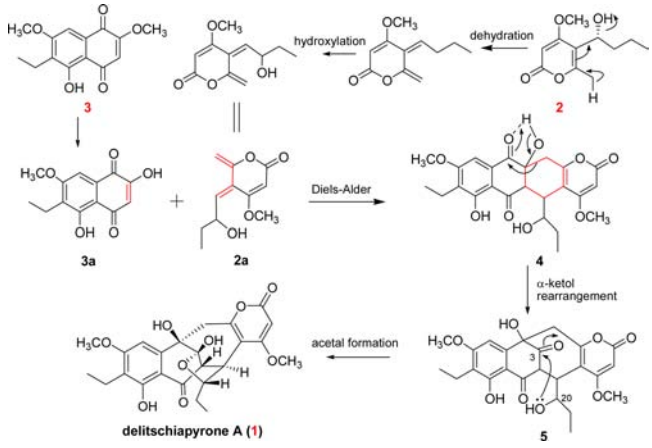


Figure 4. Calculated (black line) and experimental (red line) ECD of delitshiapyrone A (**1**).

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 pentacylic ring system in which a naphthalenone and an α-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 α-pyrone **2** and naphthoquinone **3** prompted us to postulate a biosynthetic pathway for **1** involving a Diels–Alder addition[11] followed by an α-ketol-type rearrangement,[12] 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**),[13] would lead to the key 6/6/6/6-fused tetracyclic intermediate **4**, which would then undergo an α-ketol-type rearrangement[12] 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 ^{1}H and ^{13}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*.[5] Comparison of the $[\alpha]_D$ of **2** (+15.7) with that of its analogue, macommelin-8-ol [5-(1′*S*-hydroxyethyl)-4-methoxy-6-methyl-2-pyrone] ($[\alpha]_D$ −32.6),[14] 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.[4b]

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_{50} values of 35.5, 12.9, 12.3, and 20.4 μM, 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.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support for this work from NCI (R01 CA90265), NIGMS (P41 GM09060), NSF DEB-0640996 and DEB-1045766, and the China Scholarship Council (Fellowship to J.-G.L.) is gratefully acknowledged. We thank Drs. F. Lutzoni and J. Miadlikowska, Department of Biology, Duke University, for their help with the collection of the fungal strain and Ms. P. Espinosa-Artiles of the School of Natural Resources and the

dx.doi.org/10.1021/ol502973c | *Org. Lett.* 2014, 16, 5944–5947

Environment, University of Arizona, for large-scale culturing of the fungus.

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(6) Delitschiapyrone A (**1**): pale yellow needles; $[\alpha]^{25}_{D}$ +121.0 (*c*, 0.12, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 298 (3.12) nm; CD (CH_3OH) λ_{max} ($\Delta\varepsilon$) 324 (+6.94), 279 (+6.13), 204 (−7.30) 208 (−36.79) nm; IR (KBr) ν_{max} 3451, 2967, 2933, 2851, 1694, 1629, 1580, 1457, 1409, 1383, 1307, 1248, 1133, 1081, 813, 708; HRESIMS *m*/*z* 457.1487 $[M - H]^-$ (calcd for $C_{24}H_{25}O_9$ 457.1504).

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