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 intramolecular Diels–Alder-type addition of the precursors derived from (1′R)-2’,3’,4’-dihydropyrenocine C (2) and 6-ethyl-2,7-dimethoxyjuglone (3) found to co-occur with 1 in this fungus.

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

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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 isochromenones,4b naphthoquinones,4c 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’,4’-dihydropyrenocine C (2)5 and 6-ethyl-2,7-dimethoxyjuglone (3)6 (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.

The molecular formula of delitschiapyrone A (1)6 was determined to be C24H26O9 by a combination of its HRESIMS and 13C NMR data, which required 12 degrees of unsaturation. The UV λmax 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 13C NMR spectra of 1 interpreted with the aid of DEPT, HSQC, and HMBC spectra revealed the presence of signals attributable to a chelated OH (δH 12.67), two nonchelated OH [δH 5.81 (br s) and 5.03 (br s)], two OCH3 (δH 4.00 and 3.89), two CH2, three CH3, and five CH including one aromatic (δH 7.11 (s); δC 101.3) and one olefinic [δH 5.38 (s); δC 188.6], 11 quaternary carbons of which eight are aromatic/olefinic (δC 169.4, 165.5, 161.8, 159.5, 148.6, 118.6, 113.3, and 111.6), one acetal (δC 108.4), one

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Supporting Information

Figure 1. Structure of delitschiapyrone A (1).
systems due to 16 suggested that the moieties. These data accounted for all the NMR resonances of H-5 to C-1 allowed elaboration of the previously elements (Figure 2). The HMBC correlations of H3-12/C-7, H2-11/C-6, H2-11/C-8, H-5/C-9, OH (δH 12.67)/C-9, and OCH3 (δH 3.89) to C-16 allowed the CH2 as protons of this group at δH 3.70 (J = 15.6 Hz) and 2.66 (δH, J = 15.6 Hz) showed HMBC correlations to C-3 and C-4 of the naphthalen-1(4H)-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 (δC 108.4) and C-20 (δC 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-20 revealed these to be on the same side of the furan ring, while NOESY correlations of 4-OH/H-13a (δH 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 carboxyclic 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. 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)

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

| no. | $\delta_{H}$, mult (J, Hz) | $\delta_{C}$, 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   | 111.6 C                   |                   |      |
| 8   | 117.6 C                   |                   |      |
| 9   | 148.6 C                   |                   |      |
| 10  | 162.8 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 |
| 14  | 2.66, d (15.6)            |                   | 3, 4, 10, 17, 18 |
| 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 |
| 22  | 1.78, dq (7.6, 7.2)       |                   | 2, 19, 22 |
| OMe-6 | 0.99, t (7.6)             | 10.5 CH$_3$       | 20, 21 |
| OMe-16 | 3.89, s                   | 56.5 CH$_3$       | 6 |
| OH-3  | 5.81, br s                | 57.4 CH$_3$       | 16 |
| OH-4  | 5.03, br s                |                   | 3, 4, 10, 13 |
| OH-8  | 12.67, s                  |                   | 7, 8, 9 |

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The TOCSY spectrum of 1 revealed the presence of spin systems due to −CH$_2$CH$_3$ and −CHCHCH$_2$CH$_3$ fragments (Figure 2). The HMBC correlations of H$_2$-12/C-7, H$_2$-11/C-6, H$_2$-11/C-8, H-5/C-9, OH (δ$_H$ 12.67)/C-9, and OCH$_3$ (δ$_H$ 3.89)/C-6, suggested that 1 contained an aromatic ring bearing H, OCH$_3$, CH$_2$CH$_3$, 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$_H$ 3.70), and H-2/C-19 and H$_2$-20/17/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 (δC 108.4) and C-20 (δC 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.

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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. As depicted in Figure 4, the predicted ECD for \( \text{delitschiapyrone A (1)} \) was found to be in agreement with its experimental ECD, allowing the assignment of the absolute configuration of delitschiapyrone A as \( \text{2R,3R,4R,19S,20S} \).

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 followed by an \( \alpha \)-ketol-type rearrangement, 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), would lead to the key \( 6/6/6/6 \)-fused tetracyclic intermediate 4, which would then undergo an \( \alpha \)-ketol-type rearrangement providing the \( 6/7/7/6 \) 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).

The \( ^1 \text{H} \) and \( ^{13} \text{C} \) NMR data of 2 were identical with those reported for 2',3'-dihydropyrenocine C [5-(1'-hydroxybutyl)-4-methoxy-6-methyl-2-pyrole] obtained as a racemic mixture by the catalytic reduction of pyrenocine C, a metabolite of Pyrenochaeta terrestris. Comparison of the [\( \alpha \)] of 2 (+15.7) with that of its analogue, macommelin-8-ol [5-(1'S-hydroxyethyl)-4-methoxy-6-methyl-2-pyrole] ([\( \alpha \]) = -32.6), 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.

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 \( \mu \)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.

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Notes

The authors declare no competing financial interest.

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(6) Delitschiapryone A (1): pale yellow needles; [α]α +121.0 (c, 0.12, CH3OH); UV (CH3OH) λmax (log ε) 298 (3.12) nm; CD (CH3OH) λmax (Δε) 330 (+6.94), 279 (+6.13), 204 (−7.30) 208 (−36.79) nm; IR (KBr) νmax 1455, 1409, 1383, 1307, 1248, 1133, 1081, 813, 708; HRESIMS m/z 457.1487 [M − H]− (calcd for C24H25O9 457.1504).

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