Organic LETTERS

Letter

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Opaliferin, a New Polyketide from Cultures of Entomopathogenic Fungus *Cordyceps* sp. NBRC 106954

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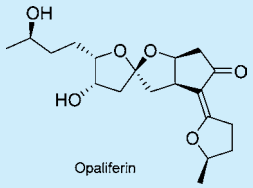
Supporting Information

ABSTRACT: Opaliferin, a polyketide with a unique partial structure in which a cyclopentanone and tetrahydrofuran were connected with an external double bond, was isolated from the insect pathogenic fungus *Cordyceps* sp. NBRC 106954. The structure and relative configuration of opaliferin were determined by spectroscopic analysis and X-ray crystallography. The absolute configuration was established by anomalous dispersion effects in X-ray diffraction measurements on the crystal of di(*p*-bromobenzoyl) ester of opaliferin. A plausible biosynthetic pathway for opaliferin is proposed.



Cordyceps species are entomopathogenic fungi that are used as a health food and traditional medicine in Asian countries. They are a rich source of secondary metabolites with a broad variety of biological and pharmacological activities in hepatic, renal, cardiovascular, immunologic, and nervous systems, as well as anticancer activity.[1]

Cordyceps sp. NBRC 106954 is a single ascospore isolated from a fruiting body occurring on a larva of cicada (*Meimuna opalifera* Walker) in Japan. We studied the metabolite of cultures of this rare fungus, *Cordyceps* sp. NBRC 106954. Here, we report the isolation and structural elucidation of a polyketide with a novel C_{19} skeleton, named opaliferin (**1**), from the insect pathogenic fungus *Cordyceps* sp. NBRC 106954 (Figure 1).

Cordyceps sp. NBRC 106954 was cultivated in 29 Erlenmeyer flasks (1 L) containing 400 mL of PSA medium for 30 days at 28 °C. The agar medium was separated from the mycelium and extracted with MeOH (Supporting Information). Water was added to the resulting residue (47.1 g), and the mixture was portioned with EtOAc and *n*-BuOH. The EtOAc-soluble extract (1.31 g) was separated by silica gel column chromatography to provide 10 fractions. Fractions 6 (72 mg) was further chromatographed on silica gel to yield 22.9 mg of opaliferin (**1**), along with known C_{10} polyketides, cephalosporolide G (**2**)[2] (2.1 mg), and a mixture of decarestrictine C_1 (**3**) and C_2 (**4**) (4.5 mg) (Figure 2).[3]

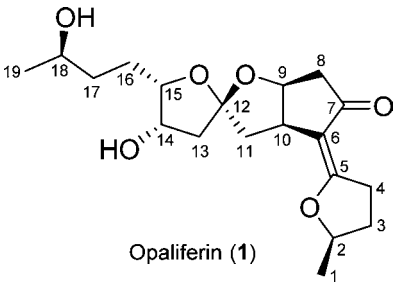


Figure 1. Structure of (+)-(2*R*,9*S*,10*R*,12*R*,14*S*,15*S*,18*R*)-opaliferin (**1**).

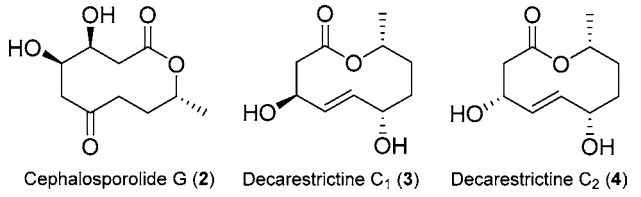


Figure 2. Structures of compounds **2**–**4**.

The structure of **1** was characterized on the basis of comprehensive mass and NMR data interpretation (Table 1). The molecular formula of **1** was determined to be $C_{19}H_{28}O_6$, as deduced by HREIMS at m/z 352.1893 $[M]^+$ (calcd 352.1900). The ^{13}C NMR and DEPT spectra indicated resonances for 19 carbons attributable to one ketone carbon (δ_c 204.1), one olefinic quaternary carbon (δ_c 170.8), two olefinic or acetal carbons (δ_c 114.3, 110.5), five oxymethine carbons (δ_c 81.9, 80.8, 76.6, 72.7, 67.8), one methine carbon (δ_c 43.0), seven methylene carbons (δ_c 45.7, 45.4, 43.8, 35.8, 30.8, 30.5, 24.8),

Received: July 27, 2014
Published: August 29, 2014

dx.doi.org/10.1021/ol502216j | *Org. Lett.* 2014, 16, 4695–4697

Table 1. ^{13}C and ^{1}H NMR Data for 1 Recorded in $CDCl_3$ at 125 and 500 MHz, Respectively

no.	δ_C	δ_H, mult (J, Hz)
1	20.5	1.36, d (6.2)
2	80.8	4.58, m
3	30.8	1.66, m
		2.22, dddd (12.6, 9.1, 6.4, 4.4)
4	30.5	2.93, dtd (18.7, 9.1, 2.3)
		3.32, dddd (18.7, 8.9, 4.4, 1.6)
5	170.8	
6	110.5	
7	204.1	
8	45.4	2.44, dd (18.8, 1.3)
		2.56, dd (18.8, 6.6)
9	76.6	4.62, m
10	43.0	3.62, m
11	43.8	2.02, dd (13.5, 5.7)
		2.64, dd (13.5, 8.7)
12	114.3	
13	45.7	2.05, dd (14.8, 1.6)
		2.49, dd (14.8, 6.2)
14	72.7	4.27, ddd (6.2, 3.4, 1.6)
15	81.9	3.92, ddd (7.1, 6.3, 3.4)
16	24.8	1.76, m
		1.80, m
17	35.8	1.58, m
		1.63, m
18	67.8	3.85, m
19	23.6	1.21, d (6.2)

and two methyl carbons (δ_c 23.6, 20.5). These assignments were fully corroborated by HSQC experiment.

The cross peaks of H_3-1/H-2/H_2-3/H_2-4, H_2-8/H-9/H-10/H_2-11 and H_2-13/H-14/H-15/H_2-16/H_2-17/H-18/H_3-19 in the $^{1}H-^{1}H$ COSY spectrum showed connectivities trough C-1 to C-4, C-8 to C-11, and C-13 to C-19 (Figure 3).

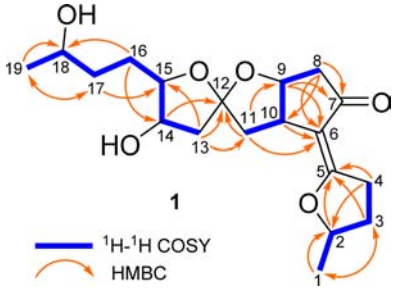


Figure 3. Selected HMBC and $^{1}H-^{1}H$ COSY corelations of opaliferin (**1**).

The HMBC correlations of H_2-11/C-12, H_2-13/C-11, C-12, and H-14/C-12 indicated that C-11 was linked with C-13 through the acetal carbon C-12 (Figure 3). The cyclopentanone moiety (C-6−C-10) was established by H-8, H-9/C-6, C-7, and H-10, H-11/C-6 correlations. The presence of tetrahydrofuran moiety (C-1−C-5) was confirmed by HMBC correlations from H-2, H_2-3, and H_2-4 to C-5. Although the correlation between H_2-4/C-6 and H-10/C-5 was not observed, the existence of double bond between carbons C-5 and C-6 was deduced on the basis of the molecular formula of **1** and chemical shifts of these carbons in ^{13}C NMR spectrum (δ_c 170.8, 110.5, respectively).

The *cis* orientation of H-9/H-10 and H-14/H-15, and therefore the relative configuration at C-9, C-10, C-14, and C-15, was determined by NOESY correlations (Figure S6, Supporting Information). The relative stereochemistry at C-2, C-12, and C-18 and the *E* configuration of the double bond between C-5 and C-6 was assigned using X-ray crystallographic analysis (Figure 4). To determine the absolute configuration of

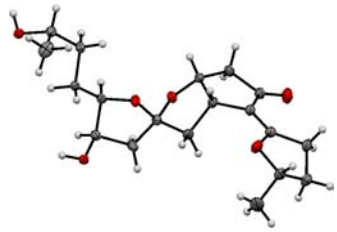


Figure 4. X-ray structure of **1**.

1, the synthesis of di(*p*-bromobenzoyl)opaliferin (**5**) was carried out. Compound **5** was formed by treating opaliferin (**1**) with *p*-bromobenzoyl chloride. Single crystals of **5** (Figure 5), suitable for single-crystal X-ray diffraction, were obtained by

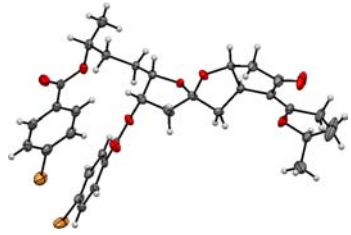


Figure 5. X-ray structure of **5**.

slow evaporation of solvents mixture (hexane−EtOAc, 3:1). The absolute configuration of all stereogenic centers was 2*R*,9*S*,10*R*,12*R*,14*S*,15*S*,18*R* as established by anomalous dispersion effects in diffraction measurements on the crystal.

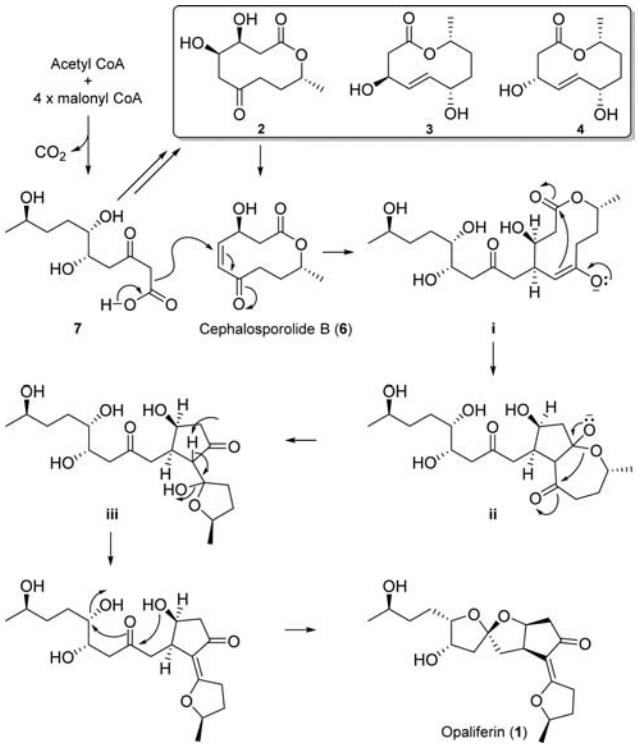
A plausible biosynthetic route to opaliferin (**1**) was proposed as shown in Scheme 1. Cephalosporolide B (**6**) can be the biosynthetic precursor for both cephalosporolide G (**2**),[2,4] C (**8**), E (**9**), F (**10**),[4,5] and other compounds with a polyketide skeleton, such as tenuipyrone (**11**)[6] and pyridomacrolidin (**12**) (Figure 2 and 6).[7] The intermediate (i) may be generated by Michael addition of **6** and (5*S*,6*S*,9*R*)-5,6,9-trihydroxy-3-oxodecanoic acid (**7**) accompanied by decarboxylation. The intermediate (i) may be cyclized to a 2*H*-cyclopent[*b*]oxepin intermediate (ii) by Claisen condensation. The rearrangement reaction and spiro-cyclization reaction of (ii) should be convert to opaliferin (**1**).

Opaliferin (**1**) was tested for antitrypanosomal and antimalarial activities. No significant inhibitory activity against *Trypanosoma brucei brucei* and *Plasmodium falciparum* was observed at 100 μM under the condition tested. Opaliferin (**1**) showed weak cytotoxicity against three tumor cell lines (HSC-2, HeLa, and RERF-LC-KJ). **1** (100 μM) inhibited 60% of HSC-2 cells proliferation, 30% of HeLa cells, and 20% of RERF-LC-KJ cells, respectively.

In conclusion, a novel polyketide, opaliferin (**1**), was isolated from entomopathogenic fungi of the genus *Cordyceps*. Opaliferin (**1**) is the second example with a unique structure in which a cyclopentanone and tetrahydrofuran were connected with an external double bond.[8] It is the first report on

dx.doi.org/10.1021/ol502216j | *Org. Lett.* 2014, 16, 4695−4697

Scheme 1. Proposed Biosynthesis of Opaliferin (1)



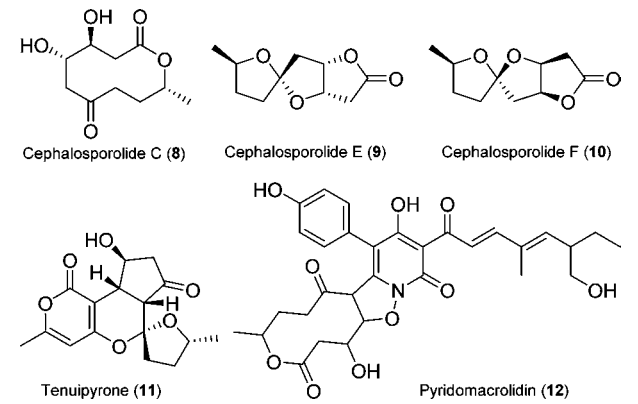


Figure 6. Structures of compounds **8**–**12**.

determination of unique structure, absolute configuration and a plausible biosynthetic pathway of opaliferin (**1**).

■ ASSOCIATED CONTENT

Supporting Information

Taxonomy analysis of *Cordyceps* sp. NBRC 106954; experimental procedure; NMR spectra for **1** and **5**; crystallographic data for **1** (CCDC 931134) and **5** (CCDC 931135) (CIF). 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

This work was supported by the Senryaku project (No. S0891078) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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dx.doi.org/10.1021/ol502216j | *Org. Lett.* 2014, 16, 4695–4697