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Tenuipyrone, a Novel Skeletal Polyketide from the Entomopathogenic Fungus, *Isaria tenuipes*, Cultivated in the Presence of Epigenetic Modifiers

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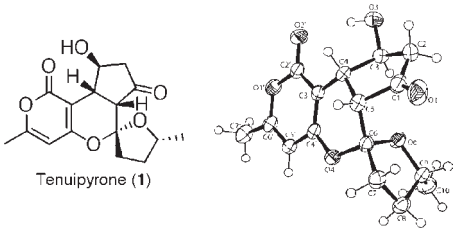
ABSTRACT



Tenuipyrone (**1**)

The concomitant addition of the histone deacetylase inhibitor and the DNA methyltransferase inhibitor to the culture medium of an entomopathogenic fungus, *Isaria tenuipes*, greatly enhanced its secondary metabolite production and led to the isolation of tenuipyrone (1), a novel polyketide with an unprecedented tetracyclic ring system bearing a spiroketal structural component, along with two known C_{10}-polyketides, cephalosporolide B (2), which is a plausible biosynthetic precursor of 1, and cephalosporolide F (3).

Filamentous fungi are an important source of pharmacologically active metabolites, and a vast diversity of natural products has been isolated from them. Fungal genome sequence analysis has revealed that fungi can potentially produce a variety of novel secondary metabolites; however, most gene clusters that code for the biosynthesis of secondary metabolites remain unexpressed under laboratory culture conditions. Hence, obtaining a variety of natural products available through these silent gene clusters has proven to be a major challenge. Epigenetic modifying agents, such as histone deacetylase (HDAC) or DNA methyltransferase inhibitors, were recently introduced as a promising approach to manipulating the fungal epigenome for the purpose of gaining access to hitherto unknown metabolites.[1,2] We applied this method to entomopathogenic fungal cultures and succeeded in obtaining three new tryptophan analogs from *Torrubiella luteorostrata*[3] and two new aromatic polyketide glycosides from *Cordyceps indigotica*[4] using SBHA (an HDAC

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inhibitor) and 5-azacytidine (a DNA methyltransferase inhibitor), respectively. Screening of the secondary metabolites produced by entomopathogenic fungi grown in the presence of these inhibitors showed that the concomitant addition of SBHA and RG-108 (another DNA methyltransferase inhibitor) to the culture medium of *Isaria tenuipes* induced significant changes in the production of secondary metabolites (Figure S1). This approach led to the isolation of tenuipyrone (**1**), a novel skeletal polyketide, together with cephalosporolides B (**2**) and F (**3**). Herein, we describe the structure elucidation of **1** by spectroscopic analysis, X-ray single crystal diffraction studies, and CD spectroscopy, and we describe a plausible biosynthetic pathway.

The culture medium (6.9 L) of *Isaria tenuipes*, which had been supplemented with SBHA (500 μM) and RG-108 (500 μM), was extracted with EtOAc. The EtOAc-soluble extract (1.9 g) was separated by silica gel column chromatography and reversed-phase HPLC to provide **1** (1.2 mg), **2** (20.1 mg), and **3** (58.5 mg) (Figure 1).[5]

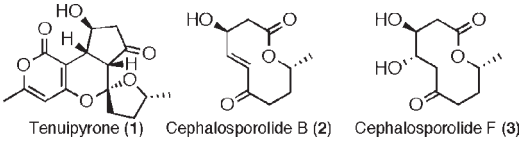

Figure 1. Structures of compounds **1**–**3**.

The molecular formula of tenuipyrone (**1**), which produced yellowish crystals, was determined to be $C_{16}H_{18}O_6$, as deduced by HREIMS at m/z 306.1109 $[M]^+$ (calcd 306.1103), requiring 8 degrees of unsaturation. The UV absorption spectrum (292 nm (log ε = 3.86)) and IR spectrum (1683 and 1584 cm^{-1}) indicated the presence of an α-pyrone moiety,[6] which was found to be trisubstituted by ^{13}C NMR and DEPT spectroscopy (δ 167.2, 162.7, 161.3, 100.9, 100.5) (Table 1).[7] In addition, one keto carbonyl, one acetal, two oxymethines, two methines, three methylenes, and two methyls were observed. The cross peaks of H_2-2/H-3, H-3/H-4, H-4/H-5, H_2-7/H_2-8, H_2-8/H-9, and H-9/H_3-10 in the $^1H-^1H$ COSY spectrum showed connectivities through C-2 to C-5 and C-7 to C-10 (Figure 2). The HMBC correlations of H-4/C-6, H_2-7/C-5, C-6, and H_2-8/C-6 indicated that C-5 was linked with C-7 through the acetal carbon C-6 (Figure 2). The 3-hydroxycyclopentanone (C-1–C-5) and tetrahydrofuran (C-6–C-10) moieties were

Table 1. ^1H and ^{13}C NMR Data of Tenuipyrone (**1**)[a]

position	δ_C, mult.	δ_H, mult. (J in Hz)
1	211.4, C	
2	46.7, CH_2	2.81 dd (19.4, 8.0, Ha)
		2.38 dd (19.4, 8.4, 1.4, Hb)
3	73.9, CH	4.62 ddd (8.8, 8.4, 8.0)
4	40.8, CH	3.18 dd (9.9, 8.8)
5	50.4, CH	2.85 d (9.9)
6	106.3, C	
7	35.1, CH_2	2.69 ddd (13.2, 12.5, 8.5, Ha)
		2.17 dd (13.2, 7.0, Hb)
8	30.9, CH_2	2.11 m (Ha)
		1.80 m (Hb)
9	79.8, CH	4.15 m
10	22.6, CH_3	1.17 d (6.2)
2′	167.2, C	
3′	100.5, C	
4′	162.7, C	
5′	100.9, CH	5.82 s
6′	161.3, C	
7′	19.8, CH_3	2.27 s
OH		5.37 s

[a] Measured at 600 MHz (^1H) and 150 MHz (^{13}C) in $CDCl_3$

deduced from the H-2, H-5/C-1, and H-9/C-6 long-range correlations. The connectivity between C-4 and C-3′ was deduced based on the HMBC correlations among H-3/C-3′ and H-4/C-2′, C-3′, C-4′. In addition, the cross peaks of H_3-7′/C-5′, C-6′, and H-5′/C-3′, C-4′, C-6′ indicated the presence of the 3,4,6-trisubstituted α-pyrone. The molecule required an additional ring to satisfy the degree of unsaturation, suggesting that the acetal carbon (C-6) was bound to C-4′ through an oxygen atom.

The relative configurations of the stereogenic centers at C-3, C-4, C-5, C-6, and C-9 of **1** were assigned by 600 MHz 1D NOE experiments in $CDCl_3$ and the value of coupling constant (Figure 2). NOE detection of Hb-2/H-4, H-5, and H-4/H-5 suggested that Hb-2, H-4, and H-5 were situated on the same side, indicating that H-4 and H-5 assumed a cis configuration. The NOE measurements of Ha-2/H-3 and the coupling constant of H-3/H-4 (8.8 Hz) revealed the anti relationship between H-3 and H-4. The spiro portion of the

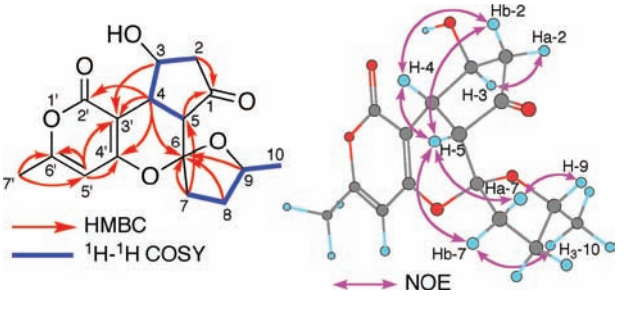

Figure 2. Selected HMBC and $^1H-^1H$ COSY correlations and key NOEs of tenuipyrone (**1**).

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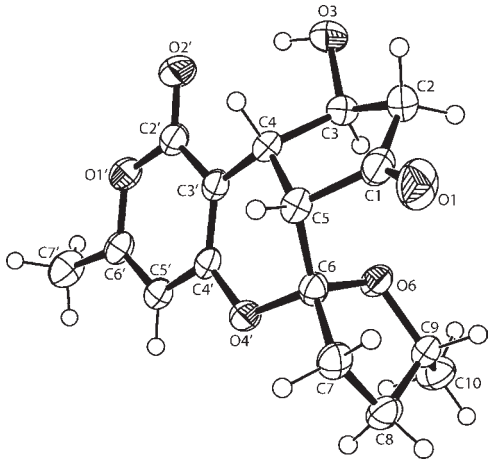


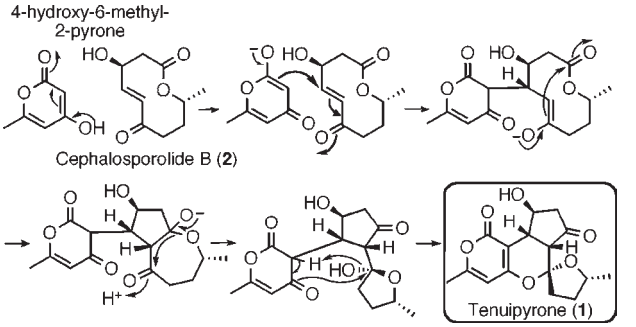
Figure 3. X-ray crystallographic structure of tenuipyrone (**1**).

structure was solved based on the proximity of C-5 and C-7, as evidenced by the NOE effects of H-5/Ha-7, Hb-7. Furthermore, the relative configuration at C-9 was deduced from the NOE enhancements of Ha-7/H-9 and Hb-7/H_3-10. The CD spectrum of **1** in EtOH showed a negative Cotton effect at 284 nm ($\Delta\varepsilon = -3.1$) for n→π^* corresponding to the saturated ketone, indicating its absolute configuration as 3*S*, 4*R*, 5*R*, 6*R*, 9*R*. The structure of **1** including its absolute stereochemistry deduced above was confirmed based on X-ray crystallographic analysis (Figure 3).

A plausible biosynthetic pathway for **1** is proposed; it is shown in Scheme 1. The unique tetracyclic ring system in **1** may be generated from 4-hydroxy-6-methyl-α-pyrone and **2** by Michael addition, followed by sequential annulations. The configurations assigned to **1** and **2** are supported by the postulated biosynthetic route.

Few studies have reported the isolation of novel fungal secondary metabolites using HDAC or DNA methyltransferase inhibitors.[2,8] This is the first demonstration that the concomitant addition of HDAC and DNA methyltransferase inhibitors can lead to the isolation of a novel skeletal

Scheme 1. Plausible Biosynthetic Pathway of Tenuipyrone (**1**)



natural product from an entomopathogenic fungus. This result suggests that chemical epigenetic methods provide access to a diversity of natural products that may be hidden in silent biosynthetic pathways.

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Supporting Information Available. Experimental procedure, figure, and full spectroscopic data, NMR spectra, and the cif file for tenuipyrone (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.