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NOTE

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17-Hydroxycyclooctatin, a Fused 5−8−5 Ring Diterpene, from *Streptomyces* sp. MTE4a

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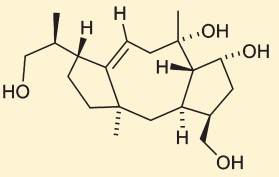
Supporting Information

ABSTRACT: A new diterpene with a fused 5−8−5 ring system was isolated from the fermentation broth of a soil actinomycete. The stereochemistry at C-15 was determined in an unusual manner using a decomposition product.



Soil actinomycetes produce a wide variety of secondary metabolites, which may provide growth advantages in their microenvironments. While bacterial secondary metabolites are widely known for antibiotic activities, some metabolites may play roles beyond the existing paradigm. For example, it is possible that bacterial metabolites mediate interactions with plants and animals, bacterial signaling, and quorum sensing.[1] In fact, recent genome sequencing of *Streptomyces griseus* IFO 13350 revealed that this well-known producer of streptomycin possesses numerous as-yet uncharacterized biosynthetic gene clusters, such as terpenoid cyclases, nonribosomal peptide synthases (NRPS), polyketide synthases (PKS), and PKS−NRPS hybrids.[2] In order to explore previously overlooked biosynthetic potential of soil bacteria, we examined soil samples from different parts of the State of New York. The study resulted in the identification of a *Streptomyces* strain that produces 17-hydroxycyclooctatin (**1**), a new member of the group of rare bacterial diterpenes with a fused 5−8−5 ring system. Here, we describe the isolation and somewhat unusual structural elucidation of **1**.

Soil samples were collected from different places in New York State, including Lake Oakland, Phoenicia, and Bayside. Sixteen different strains were isolated. LC/MS-based screening of culture supernatants indicated the production of secondary metabolites by one strain, MTE4a, which was identified as a *Streptomyces* strain based on the sequence of 16S rRNA. The culture broth was extracted with ethyl acetate. The crude extract was fractionated by silica gel chromatography, followed by HPLC using a C-18 column, to obtain 17-hydroxycyclooctatin (**1**) as a colorless solid.

The molecular formula of the purified material was deduced to be $C_{20}H_{34}O_4$ on the basis of the molecular ions observed in the negative HRESIMS: namely, *m*/*z* 372.2155 $[M + Cl]^-$ (calcd 373.2151), *m*/*z* 383.2446 $[M + HCOO]^-$ (calcd 383.2439), and *m*/*z* 451.2319 $[M + CF_3COO]^-$ (calcd 451.2313). The positive HRESIMS, on the other hand, showed only dehydrated ions at *m*/*z* 321.2425 $[M + H - H_2O]^+$ (calcd 321.2424) and *m*/*z* 303.2318 $[M + H - 2(H_2O)]^+$ (calcd 303.2319), suggesting the presence of at least two hydroxy groups. The ^1H, ^{13}C, DEPT, and HSQC spectra in chloroform-*d* were then used to identify 20 carbon units, which included two olefinic carbons (δ_C 119.6 and 154.0 ppm), four oxygen-bearing carbons (δ_C 77.2, 75.3, 65.9, and 63.5 ppm), and three methyl carbons (δ_C 26.7, 25.1, and 17.1 ppm). HMBC (10 Hz) correlations from the three methyl groups, followed by COSY analysis, led to the assembly of individual carbon units into larger fragments except for a methylene carbon at 24.8 ppm. Further analyses of HMBC unequivocally led to the elucidation of a diterpene framework with a fused 5−8−5 ring system (Figure 1 and Table 1).

Relative stereochemistry was then characterized on the basis of NOESY (Figure 1). NOESY correlations on the β-face (H-1_b/H-6; H-6/H_3-19; H_3-19/H-9) as well as those on the α-face (H-2/H-8_a/H_3-20) suggested a distinct U-shaped conformation of the central eight-membered ring as well as the ring junctions as shown in Figure 1. The relative stereochemistry at C-15, however, could not be conclusively determined due to the conformational flexibility between C-11 and C-15.

Assignment of the C-15 configuration, however, was accomplished in an unusual manner using a decomposition product of **1** in chloroform-*d*. While the NMR sample was stored at 4 °C for one week, a fraction of **1** turned into another compound (**1′**) probably because of the trace amount of acid in chloroform-*d* (Figure 2). Although many NMR signals of this decomposition product were buried under the signals of **1**, it was possible to identify the ^1H-signals of three methyl groups (H_3-16′, H_3-19′, and H_3-20′) and two oxygenated methylene groups (H-17_a′, H-17_b′, and H-18_b′) (Figure 2). A close examination of HMBC revealed that H_3-20′ and H-17_a′ of **1′** showed correlations to a carbon at ∼96

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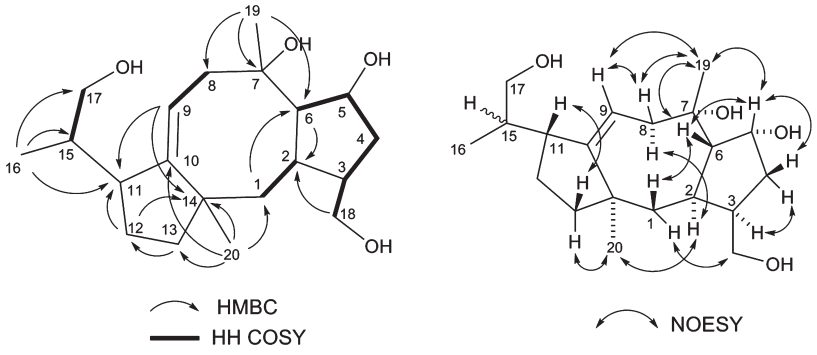

Figure 1. Structural elucidation of **1**. Shown are key COSY, HMBC, and NOESY correlations. The relative stereochemistry of C-15 could not be conclusively determined due to the rotational flexibility around the C-11/C-15 bond.

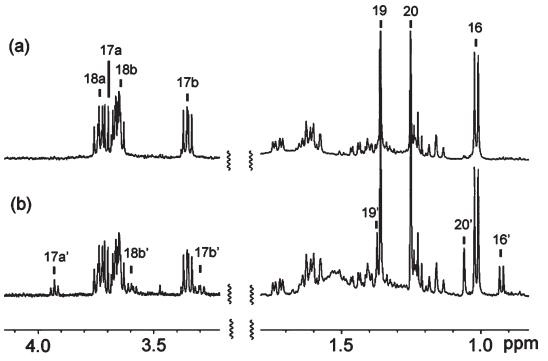

Figure 2. Decomposition of **1** in chloroform-*d*. (a) Initial ^{1}H NMR. (b) After storage for 1 week at 4 °C.

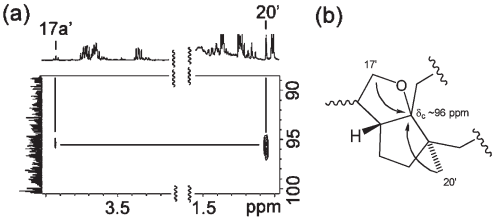

Figure 3. Structural analysis of **1′**. (a) HMBC correlations to a carbon at δ_C ~96 ppm were observed from H_3-20′ and H-17$_a$′. (b) Cyclized structure consistent with the observed HMBC signals. ChemBioDraw Ultra 11 estimated δ_C ~100 ppm for the oxygenated quaternary carbon of this cyclized structure.

ppm (Figure 3a). These HMBC correlations were consistent with a cyclized structure, in which the ^{13}C NMR chemical shift of the oxygenated quaternary carbon is estimated to be ~100 ppm according to ChemBioDraw Ultra 11 (Figure 3b). The NOESY spectrum was then reanalyzed to characterize the C-15′ stereochemistry of this cyclized compound. The analysis revealed that both H_3-20′ and H_3-16′ had NOESY correlations to H-17$_b$′ but not to H-17$_a$′ (Figure 4a). These results suggested the partial structure of **1′** as shown in Figure 4b, in which H_3-16′ was positioned closer to H-17$_b$′ than to H-17$_a$′. Thus, the relative stereochemistry of C-15 in **1** was determined as shown in Figure 5. In order to confirm the reproducible production of **1** by MTE4a as well as its chemical structure, the isolation of **1** from the culture broth was repeated. NMR analyses of **1** in methanol-d_4 (see Supporting Information), in which **1** does not decompose, led to the same structure as shown in Figure 5.

It is noted that bacterial production of terpenoids with a fused 5−8−5 ring framework is rare. Such framework is more commonly produced by fungi and insects.[3] The only other example of the bacterial production of a member of this class of compounds is cyclooctatin (**2**) (Figure 5), a lysophospholipase inhibitor from *Streptomyces melanosporofaciens* MI614-43F2.[4,5] The elucidated structure of **1** closely resembles cyclooctatin. In fact, the ^{13}C NMR signals of the two compounds are almost identical except for the 17-position (Table 1). The close structural similarity suggests that the two compounds are produced through the same biosynthetic pathway in the respective producing strains,[6] but strain MTE4a introduces a hydroxy at the C-17 position.

If we consider the typical soil environment, which can turn acidic (pH 5−6), the production of an acid-sensitive compound by soil bacteria is a peculiar finding. Although our preliminary studies suggest that **1** has weak antibacterial activity against *Staphylococcus aureus* (Figure S1), its true biological activities may be revealed after acid-catalyzed transformation.

In conclusion, we isolated and determined the structure of 17-hydroxycyclooctatin (**1**) from *Streptomyces* sp. MTE4a. It is interesting to note that due to its chemical structure and reactivity it undergoes facile transformation under a mildly acidic environment.

EXPERIMENTAL SECTION

General Experimental Procedures. NMR spectra were recorded on a Brüker Avance 500 MHz spectrometer equipped with a dual [^{13}C, ^{1}H] CryoProbe. Data were acquired and processed with the Brüker XWIN-NMR software package. LC/MS analysis was carried out with an Agilent Technologies 6210 time-of-flight mass spectrometer equipped with an Agilent Technologies 1200 capillary HPLC system. Chromatography was performed on a Zorbax 0.5 × 150 mm SB-C18 column (#5064-8256) using water containing 0.1% formic acid and 50 μM ammonium formate (solvent A) and methanol containing 0.1% formic acid and 50 μM ammonium formate (Ssolvent B) at a flow rate of 12 μL/min. The gradient program was as follows: 10% B (0−2 min), 10−100% B (2−20 min), 100% B (20−50 min). The temperature of the column was held at 40 °C for the entire run. Sample ionization was accomplished using an Agilent Technologies Electrospray source with data collection in both positive and negative ion modes. Ionization source parameters were the following: nebulizer pressure of 20 psi, drying gas temperature of 300 °C, drying gas flow rate of 8.0 L/min, and capillary voltage of 3500 V. The mass spectrometer was set to acquire data with a fragmentor voltage of 165 V. A mass range of 100 to 3200 m/z was scanned using 10 000 transients per scan. All solvents for purification were in the HPLC grade and were purchased from VWR and

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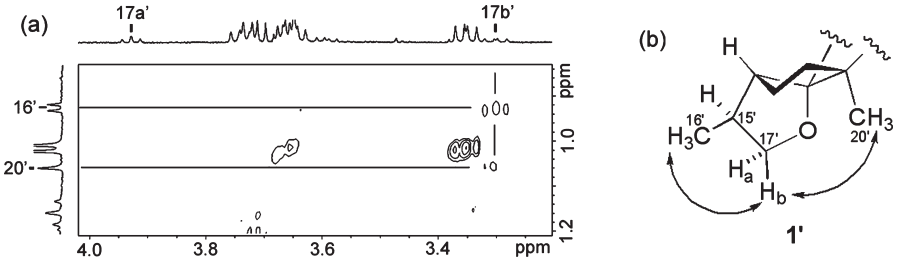

Figure 4. Characterization of C-15′ relative stereochemistry. (a) NOESY correlations were observed to H-17$_b$′ from H$_3$-16′ and H$_3$-20′. (b) Partial structure of **1′** consistent with the NOESY data.

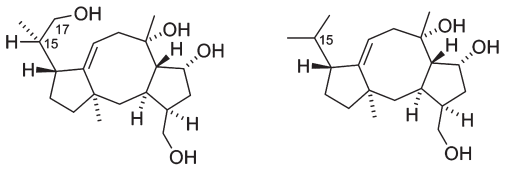

Figure 5. Structure of **1** from *Streptomyces* sp. MTE4a and its known congener, cyclooctatin (**2**), from *S. melanosporofaciens* MI614-43F2.

Table 1. ^{13}C NMR Data[a] of 17-Hydroxycyclooctatin (1) and Cyclooctatin (2)

	1	1	2
position	δ_C ($CDCl_3$)	δ_C (CD_3OD)	δ_C (CD_3OD)
1	44.5	45.5	45.6
2	34.8	35.8	35.8
3	44.4	44.9	44.9
4	39.2	39.7	39.7
5	75.3	75.7	75.7
6	57.0	57.9	58.0
7	77.2	78.4	78.4
8	41.9	42.2	42.2
9	118.8	119.6	119.1
10	153.1	154.0	154.5
11	46.0	45.9	45.9
12	45.7	46.6	46.6
13	24.8	25.3	24.3
14	51.2	52.3	55.1
15	37.4	38.8	30.2
16	17.1	17.3	17.8
17	65.9	65.6	22.5
18	63.5	63.4	63.4
19	26.7	26.7	26.7
20	25.1	25.9	25.2

[a] Recorded at 125 MHz.

Fisher Scientific. Unless specified otherwise, all other chemicals and reagents were obtained through Fisher Scientific and used without further purification.

MTE4a Collection and Culture. MTE4a was isolated from the soil sample obtained from Phoenicia, NY. Glycerol-yeast extract agar supplemented with cyclohexamide (50 mg/L) was used for the isolation of actinomycete strains. Purification of the isolated culture was performed using soy flour mannitol (SFM) agar (soy flour 20 g/L; mannitol 20 g/L; agar 20 g/L). Then 50 mL of Difco nutrient broth (3 g of beef extract, 5 g of peptone per liter) was inoculated with spores and shaken at room temperature at 200 rpm for 3 days. On the third day 10 mL of the inoculum was added to 125 mL of sterile MTF1 (250 mL flasks). MTF1 medium was prepared by mixing yeast extract (1 g), tryptone (10 g), K_2HPO_4 (0.5 g), glycerol (8 g), and dextrose (2 g) in 1 L of distilled water (adjusted to pH 7.1−7.3). The cultures were grown at room temperature and 200 rpm for seven days, and the supernatant was collected.

Purification of 1. Seven liters of MTE4a culture was centrifuged for 30 min at 6000 rpm at 4 °C. The supernatant (∼6 L) was then extracted with the 6 L of EtOAc. The extract was separated by silica gel chromatography using a step gradient from CH_2Cl_2 to 10% MeOH in CH_2Cl_2. The fraction containing **1** was eluted with 10% MeOH in CH_2Cl_2. The fraction was further purified with RP-HPLC (C_{18}, 4.6 × 150 mm, 1 mL/min, 20−80% MeOH(aq) over 30 min). HPLC eluent was monitored with a photodiode array (PDA) detector and evaporative light scattering detector (ELSD). The major ELSD peak at 22.5 min was collected and dried to give a colorless and clear solid of **1** (2 mg).

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra and a preliminary characterization of antimicrobial activity of **1** are available free of charge via the Internet at http://pubs.acs.org.

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■ DEDICATION

Dedicated to Dr. Koji Nakanishi of Columbia University for his pioneering work on bioactive natural products.

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