J. Antibiot. 61(7): 415–419, 2008

ORIGINAL ARTICLE

THE JOURNAL OF ANTIBIOTICS

Tetrahydrobostrycin and 1-Deoxytetrahydrobostrycin, Two New Hexahydroanthrone Derivatives, from a Marine-derived Fungus *Aspergillus* sp.

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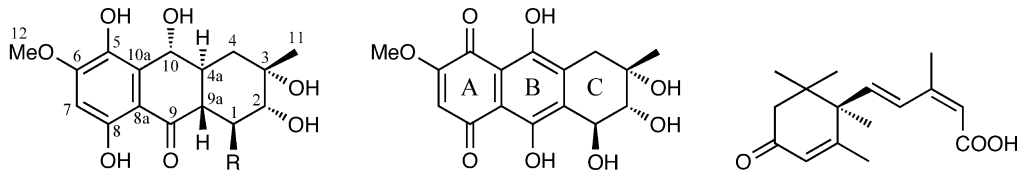
Received: March 18, 2008 / Accepted: June 14, 2008

Abstract Two new hexahydroanthrones, tetrahydrobostrycin (**1**) and 1-deoxytetrahydrobostrycin (**2**), were isolated from a marine-derived fungus *Aspergillus* sp. strain 05F16 collected at the coral reef of Manado, Indonesia, together with bostrycin and abscisic acid. The structures of new compounds were determined on the basis of their spectral data. Compound **1** showed weak antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* and **2** against *S. aureus*.

Keywords marine-derived fungus, *Aspergillus* sp., tetrahydrobostrycin, 1-deoxytetrahydrobostrycin, hexahydroanthrone, bostrycin, abscisic acid

Introduction

Marine-derived fungi have proven to be a prolific source of new bioactive secondary metabolites [1~3]. Diverse chemical structures with interesting biological activities, such as cytotoxicity against cancer cell lines, antimicrobial activity, and inhibition of specific enzyme activities, have been reported in scientific journals [1~3]. Therefore, we have further investigated bioactive secondary metabolites of marine-derived fungi isolated from tropical coral reefs and obtained two new hexahydroanthrone derivatives, 1,2,3,5,8,10-hexahydroxy-6-methoxy-1,2,4,4a,9a,10-hexahydroanthracen-9-one (named as tetrahydrobostrycin, **1**) and 2,3,5,8,10-pentahydroxy-6-



tetrahydrobostrycin (**1**): R = OH
1-deoxytetrahydrobostrycin (**2**): R = H

bostrycin (**3**)

abscisic acid (**4**)

Fig. 1 Structures of tetrahydrobostrycin (**1**), 1-deoxytetrahydrobostrycin (**2**), bostrycin (**3**), abscisic acid (**4**).

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methoxy-1,2,4,4a,9a,10-hexahydroanthracen-9-one (named as 1-deoxytetrahydrobostrycin, **2**), from *Aspergillus* sp. collected in Manado, Indonesia, together with a red pigment bostrycin (**3**) [4～10], and the plant hormone abscisic acid (**4**). We describe herein the isolation, structure elucidation, and biological activity of two new compounds **1** and **2**.

Materials and Methods

General Experimental Procedures

NMR spectra were measured on a JEOL JNM-AL-400 or JNM-LA-600 NMR spectrometer in DMSO-d_6 (δ_H 2.49, δ_C 39.5) or CD_3OD (δ_H 3.30, δ_C 49.0). Mass spectra were obtained by a JEOL JMS-MS 700 mass spectrometer (EI or FAB mode with *m*-nitrobenzyl alcohol or glycerol as the matrix). UV and IR spectra were recorded on a Hitachi U-3310 spectrophotometer and a Perkin-Elmer Spectrum One FT-IR spectrometer, respectively. Optical rotations were recorded with a JASCO DIP-370 digital polarimeter. HPLC was performed using a HITACHI Pump L-2130 equipped with a UV Detector L-2400.

Producing Organism

The fungus 05F16 was isolated from an unidentified alga collected in the coral reef at Manado, Indonesia. The alga was smashed with sterile seawater in a mortar and the resulted liquid (0.2 ml) was applied on an agar plate (1/10 YSA, 90% seawater). The fungus grown on the agar plate was isolated and identified as *Aspergillus* sp. from the shapes and colors of conidia and mycelia. The strain 05F16 was cultured in twelve 500-ml Erlenmeyer-flasks containing each 150 ml of 1/2 PD medium (50% natural seawater) [11] for about three weeks at 20°C.

Extraction and Isolation

The culture broth of 05F16 (1,800 ml) was filtered and extracted with EtOAc (3×900 ml). A portion (100 mg) of the EtOAc extract (520 mg) was subjected to HPLC separation (column, Pegasil ODS, 10 mm×250 mm; flow rate, 2.0 ml/minute; detection, UV 280 nm) with MeOH-H_2O=52 : 48 to give compound **1** (20.0 mg), abscisic acid (**4**, 12.0 mg), and a mixture of **2** and **3** (29.0 mg), which was

Table 1 ^{1}H- (600 MHz) and ^{13}C- (100 MHz) NMR data for compound **1**

C#	^{13}C (δ)[a]	^{1}H (δ, mult, *J* in Hz)[a]	^{1}H (δ, mult, *J* in Hz)[b]
1	69.7	3.85, ddd, 13.2, 8.8, 2.9	4.07, dd, 9.5, 9.2
2	77.9	3.05, dd, 8.8, 5.4	3.27, d, 9.2
3	70	—	—
4	39.6	(*ax.*) 1.30, dd, 13.2, 12.5	(*ax.*) 1.42, dd, 13.6, 11.7
		(*eq.*) 2.10, dd, 13.2, 3.9	(*eq.*) 2.32, dd, 13.6, 3.7
4a	39.6	2.20, m, 12.5, 9.8, 9.3, 3.9	2.28, m, 13.2, 11.7, 9.5, 3.7
5	137.5	—	—
6	155.6	—	—
7	99.2	6.49, s	6.46, s
8	157.2	—	—
8a	108.1	—	—
9	203.8	—	—
9a	52.1	2.42, dd, 13.2, 9.3	2.38, dd, 13.2, 9.5
10	71.8	4.76, dd, 9.8, 6.8	4.80, d, 9.5
10a	127.7	—	—
11	27.1	1.15, s	1.31, s
12	56	3.83, s	3.90, s
1-OH	—	4.46, d, 2.9	—
2-OH	—	4.56, d, 5.4	—
3-OH	—	4.08, s	—
5-OH	—	9.28, s	—
8-OH	—	12.22, s	—
10-OH	—	6.37, d, 6.8	—

[a] Measured in DMSO-d_6. [b] Measured in CD_3OD.

further separated by HPLC (same column) with MeOH-H_2O=38 : 62 to yield compound **2** (6.8 mg) and bostrycin (**3**, 10.8 mg).

Tetrahydrobostrycin

$[\alpha]_D^{18}$ −116.6° (*c* 0.8, MeOH). UV λ_{max}^{MeOH} nm (log ε) 244 (3.97), 284 (3.88), 355 (3.63). IR ν_{max} (KBr) cm^{-1} 3428, 2978, 2928, 1627, 1495, 1443, 1385, 1281, 1245, 1204, 1157, 1072, 1053, 1034. ^{1}H-NMR (600 MHz in DMSO-d_6 and in CD_3OD) and ^{13}C-NMR (100 MHz in DMSO-d_6): see Table 1. High-resolution (HR) FAB-MS *m/z* 341.1232 $[(M+H)^+$, Calcd for $C_{16}H_{21}O_8$, 341.1236].

1-Deoxytetrahydrobostrycin

$[\alpha]_D^{18}$ −69.3° (*c* 0.4, MeOH). UV λ_{max}^{MeOH} nm (log ε) 245 (sh), 280 (3.28), 350 (3.01). IR ν_{max} (KBr) cm^{-1} 3436, 2976, 2924, 1630, 1498, 1441, 1385, 1278, 1226, 1201, 1152, 1070, 1054. ^{1}H-NMR (600 MHz in DMSO-d_6 and in CD_3OD) and ^{13}C-NMR (100 MHz in DMSO-d_6): see Table 2. HRFAB-MS *m/z* 325.1296 $[(M+H)^+$, Calcd for $C_{16}H_{21}O_7$, 325.1288].

Antimicrobial Assay

Two new compounds **1** and **2** were tested for antimicrobial activity against *Staphylococcus aureus* IAM 12544T (16 hours at 37°C), *Escherichia coli* IAM 12119T (16 hours at 37°C), *Saccharomyces cerevisiae* IAM 14383T (40 hours at 25°C), and *Mucor hiemalis* IAM 6088 (40 hours at 25°C). Test compounds were dissolved in MeOH or EtOH and 40 μl of each solution was absorbed on a disc (8 mm in diameter; 25, 50, and 100 μg/disc). After incubation, diameters of the inhibition zones were measured.

Results and Discussion

The producing fungus was isolated from an unidentified alga collected in the coral reef at Manado, Indonesia. The culture broth and EtOAc extract of strain 05F16 showed red color, and four chemical constituents (**1**∼**4**) were isolated from the EtOAc extract. Compounds **1** and **2** showed a pale-yellow color, and **3** was a red pigment whose structure was assigned from its spectroscopic data and by comparison with the reported values for bostrycin [4∼7].

Table 2 ^{1}H- (600 MHz) and ^{13}C- (100 MHz) NMR data for compound **2** in DMSO-d_6

C#	^{13}C (δ)[a]	^{1}H (δ, mult, *J* in Hz)[a]	^{1}H (δ, mult, *J* in Hz)[b]
1	29.2	(*ax.*) 1.48, ddd, 12.4, 12.2, 12.0	(*ax.*) 1.66, ddd, 12.8, 12.1, 11.7
		(*eq.*) 2.05, ddd, 12.2, 5.6, 4.0	(*eq.*) 2.29, ddd, 12.8, 4.4, 4.0
2	73.2	3.25, ddd, 12.0, 6.3, 5.6	3.44, dd, 11.7, 4.4
3	69.4	—	—
4	40.7	(*ax.*) 1.20, dd, 13.2, 12.0	(*ax.*) 1.35, dd, 13.9, 11.7
		(*eq.*) 2.15, dd, 13.2, 3.8	(*eq.*) 2.35, dd, 13.9, 4.0
4a	40.7	2.05, m, 12.2, 12.0, 10.0, 3.8	2.16, m, 12.8, 11.7, 10.3, 4.0
5	137.6	—	—
6	155.5	—	—
7	99.2	6.48, s	6.46, s
8	157.5	—	—
8a	107.6	—	—
9	202.7	—	—
9a	45.8	2.44, ddd, 12.4, 12.2, 4.0	2.40, ddd, 12.8, 12.1, 4.0
10	71.9	4.69, br d, 10.0	4.76, d, 10.3
10a	127.3	—	—
11	26.9	1.15, s	1.30, s
12	55.9	3.81, s	3.89, s
2-OH	—	4.47, d, 6.3	—
3-OH	—	3.86, s	—
5-OH	—	9.49, br s	—
8-OH	—	12.67, s	—
10-OH	—	6.51, br s	—

[a] Measured in DMSO-d_6. [b] Measured in CD_3OD.

Compound **4** was identified as abscisic acid, the plant hormone, from its spectroscopic data. Bostrycin (**3**) was first isolated from *Bostryconema alpestre* [4, 5] and then from *Nigrospora oryzae* [8], *Arthrinium phaeospermum* [9], and *Alternaria eichhorniae* [10]. Antibacterial and phytotoxic activities have been described in these papers. The predominant tautomer and absolute configuration of bostrycin were determined as **3** by syntheses [6, 7].

Compound **1** showed the $(M+H)^+$ ion at *m/z* 341.1232 in the HRFAB mass spectrum. The molecular formula, $C_{16}H_{20}O_8$, was deduced from the HRFAB-MS and NMR data (Table 1). The ^1H and ^{13}C signals of **1** were assigned by the analysis of 2D (^1H-^1H COSY, HMQC, and HMBC) and DEPT spectra. The ^{13}C-NMR spectrum of **1** revealed the presence of 16 carbon signals ascribed to a methyl, a methoxy, a methylene, two methine, three hydroxymethine, a hydroxylated quaternary carbon atom, a carbonyl, and six aromatic carbons. Six OH signals were detected in the ^1H-NMR spectrum of **1**. The connectivity of 2-1-9a-4a-(4)-10 was determined from the ^1H-^1H COSY spectrum of **1**, which also showed correlations between H-1 and 1-OH, H-2 and 2-OH, and H-10 and 10-OH (Fig. 2). The skeletal structure of **1** was assigned by the interpretation of the HMBC data (Fig. 2).

The relative stereochemistry of **1** was determined from ^1H coupling constants and NOESY data. Relatively large coupling constants observed between H-1 and H-2 (J=8.8 Hz), H-1 and H-9a (13.2), H-4*ax* and H-4a (12.5), H-4a and H-9a (9.3), and H-4a and H-10 (9.8) suggested that the orientations of these hydrogens are axial or psudoaxial. These orientations were confirmed by the analysis of NOESY spectrum of **1**. Key NOESY correlations were detected between H-1 and H-4a, H-2 and H-4*ax*, H-2 and H-9a, H-4*ax* and H-9a, H-4*ax* and H-10, H-9a and H-10, and H_3-11 and H-4*eq*. Consequently, the structure of **1** was assigned as shown in Fig. 1. Compound **1** was a tetrahydro-derivative of bostrycin (**3**) and named as tetrahydrobostrycin. The stereochemistry of **1** shown in Fig. 1 was illustrated according to the absolute structure of bostrycin (**3**) [7].

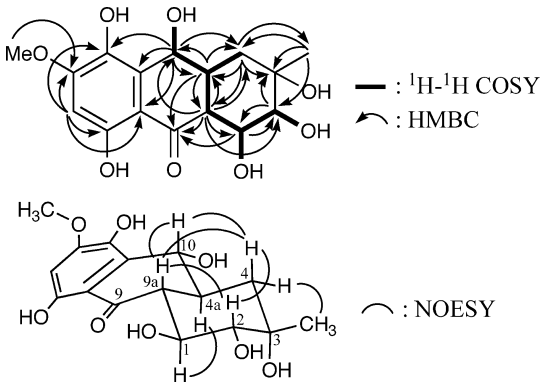

Fig. 2 ^1H-^1H COSY, HMBC, and key NOESY correlations of compound **1**

Compound **2** showed similar ^1H- and ^{13}C-NMR spectra as those of **1**, except for the lack of an OH group in the spectra of **2**. The molecular formula of **2** was determined from HRFAB-MS and NMR data as $C_{16}H_{20}O_7$, which corresponded to the difference of one oxygen (16 Da) in the molecular formula (weight) between **2** and **1**. Therefore, compound **2** was suggested to be a deoxy-derivative of **1**. ^1H- and ^{13}C-NMR data for **2** (Table 2) were assigned by the analysis of ^1H-^1H COSY, DEPT, HMQC, and HMBC spectra. The ^1H-^1H COSY spectrum of **2** revealed correlations between H-2 and 2-OH, H-2 and H_2-1, H_2-1 and H-9a, H-9a and H-4a, H-4a and H_2-4, H-4a and H-10, and H-10 and 10-OH. The HMBC spectrum of **2** gave similar correlations that observed in the HMBC spectrum of **1**. These NMR data clearly showed that the hydroxyl group at the C-1 position in **1** was replaced by a hydrogen in **2**. Coupling constants between H-1*ax* and H-2 (J=12.0 Hz), H-1*ax* and H-9a (12.4), H-4*ax* and H-4a (12.0), H-4a and H-9a (12.2), and H-4a and H-10 (10.0) observed in the ^1H-NMR spectrum of **2** were similar to those of **1**. Moreover, NOESY correlations were detected between H-1*ax* and H-4a, H-2 and H-4*ax*, H-2 and H-9a, H-4*ax* and H-9a, H-4*ax* and H-10, H-9a and H-10, and H_3-11 and H-4*eq*. Thus, the structure of **2** was assigned as a 1-deoxy derivative of **1** and named as 1-deoxyteterahydrobostrycin (Fig. 1).

Compound **1** inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* at 100 μg/disc with the inhibition zones of 15 and 9.2 mm in diameter, respectively. Compound **2** was active against *S. aureus* (12 mm at 100 μg/disc). The growth of *Saccharomyces cerevisiae* and *Mucor hiemalis* was not affected by these compounds at 100 μg/disc. It is interesting that the presence of an OH group at C-1 is important for the antibacterial activity of **1** against *E. coli*, although the antibacterial activity of these compounds are very weak. The 5-deoxy derivative of **2** (tetrahydroaltersoranol B) has been isolated from a plant pathogenic fungus *Alternaria solani* [12, 13] and showed no apparent antimicrobial activity against Gram-positive bacteria at 50 μg/ml by the broth dilution method [13]. An epimer at the 10 position of **1** has recently been isolated from the mangrove endophytic fungus *Halorosellinia* sp. [14]. The IC_{50} value of this compound against two human nasopharyngeal epidermoid tumor cell lines (KB and KBv200) was above 50 μg/ml [14].

Bostrycin (**3**) has a tetrahydroanthraquinone skeleton.

Related tetrahydroanthraquinone derivatives were produced by fungi such as *Alternaria eichhorniae* [10], *Alternaria porri* [15], *Alternaria solani* [12, 16～19], *Auxarthron umbrinum* [20], *Chrysosporium queenslandicum* [21], *Dactylaria lutea* [22, 23], *Dermocybe* sp. [24], *Phomopsis juniperovora* [25], *Pleospora* sp. [26], and *Stemphylium botryosum* [27]. The antimicrobial, antiprotozoal, phytotoxic, and cytotoxic activities of these compounds were described by the authors of these papers. The hexahydroanthronol derivatives including two new compounds **1** and **2** exhibited very weak or no apparent activity in the same and similar bioassays. Therefore, the quinone structure will be necessary for their bioactivities.

Acknowledgements This work was supported in part by Grant-in-Aid for Scientific Research on Priority Areas 17035029 from The Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan to M. N. We thank Dr. Magie M. Kapojos for her generous help during the sample collection at Manado.

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