NOTE

JBIR-59, a new sorbicillinoid, from a marine-derived fungus *Penicillium citrinum* SpI080624G1f01

Jun-ya Ueda¹, Junko Hashimoto¹, Shigeki Inaba², Motoki Takagi¹ and Kazuo Shin-ya³

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The marine environment has recently been described as a source of novel chemical diversity for drug discovery, as many bioactive substances are isolated from marine organisms such as phytoplankton, algae, sponges, tunicates and mollusks.^{1,2} Microorganisms, especially fungi, from marine habitats also constitute a promising untapped resource of novel compounds and are receiving special attention.^{3–5} We have recently discovered novel compounds, namely sesquiterpenes JBIR-27 and JBIR-28,⁶ aspochracin derivative JBIR-15⁷ and glycosyl benzenediols JBIR-37 and JBIR-38,⁸ from marine-derived fungi. Therefore, we attempted to isolate fungi from a marine sponge, Demospongiae, to obtain novel substances from the fungal culture. In the course of our screening program for discovering novel compounds from marine-derived fungi, we isolated a new compound termed JBIR-59 (**1**, Figure 1a) together with six known compounds, that is, redoxcitrinin,⁹ bisorbibutenolide,¹⁰ bisvertinolone,¹¹ trichodimerol¹² and sclerotinin A and B,¹³ from the mycelial extract of *Penicillium citrinum* SpI080624G1f01. We report herein the fermentation, isolation and, in brief, the biological activity of **1**.

Penicillium citrinum SpI080624G1f01 was isolated from the marine sponge, Demospongiae, collected offshore of Ishigaki island, Okinawa Prefecture, Japan. SpI080624G1f01 was cultivated in 50 ml test tubes containing 15 ml of potato dextrose broth (24 g l⁻¹ potato dextrose; BD Biosciences, San Jose, CA, USA). The test tubes were shaken on a reciprocal shaker (355 r.p.m.) at 27 °C for 3 days. Aliquots (5 ml) of the culture were transferred to 500-ml Erlenmeyer flasks containing 15 g of brown rice (Hitomebore, Miyagi, Japan), 30 mg of Bacto-yeast extract (BD Biosciences), 15 mg of sodium tartrate, 15 mg of potassium hydrogenphosphate and 45 ml of water; further, the aliquots were incubated in static culture at 27 °C for 14 days.

The mycelium (gathered from 10 flasks) was extracted with 80% aq. Me₂CO. After concentration *in vacuo*, the aqueous concentrate was

extracted with EtOAc. The obtained organic layer was dried over anhydrous Na₂SO₄ and evaporated to dryness. The dried residue was washed with *n*-hexane–CHCl₃ (6:1), and its insoluble fraction was subjected to normal-phase medium-pressure liquid chromatography (Purif-Pack SI 60 μm, Moritex, Tokyo, Japan) and eluted with a stepwise solvent system of CHCl₃–MeOH (100:0, 99:1, 98:2, 95:5, 90:10 and 50:50, successively). The CHCl₃-eluate afforded redoxcitrinin, whereas the CHCl₃–MeOH (99:1–98:2)-eluted fraction was chromatographed on reversed-phase medium-pressure liquid chromatography (Purif-Pack ODS 100 μm, Moritex) to yield bisorbibutenolide, bisvertinolone, trichodimerol and sclerotinin A and B. A portion (20 mg) of the CHCl₃–MeOH (98:2)-eluate (134 mg) was purified by preparative reversed-phase HPLC using an XBridge Prep C₁₈ column (5.0 μm OBD, 19 i.d. × 150 mm; Waters, Milford, MA, USA) developed with 55% MeOH–H₂O (flow rate: 10 ml min⁻¹) to yield **1** (12.3 mg, retention time: 17.2 min).

Compound **1** was obtained as a yellow amorphous solid ([α]_D²⁵ –350°, *c* 0.1, in MeOH; UV λ_{max} 221, 263 and 387 nm, in MeOH), and its molecular formula was determined to be C₂₃H₂₈O₇ by HR-ESI-MS (*m/z* 417.1910, (M+H)⁺; calcd for C₂₃H₂₉O₇, 417.1913). The IR spectra (ν_{max} 1658 cm⁻¹) of **1** showed the presence of a conjugated ketone. The direct connectivity between each proton and carbon was established by the HSQC spectrum, and the ¹³C and ¹H NMR spectral data for **1** are listed in Table 1. A planar structure was established by double-quantum filtered COSY spectrum together with constant time HMBC¹⁴ spectrum as follows.

An allylic coupling between the methyl protons 10-H₃ (δ_H 2.01) and an olefinic proton 2-H (δ_H 5.64) was observed in the double-quantum filtered COSY spectrum, as shown in Figure 1b. In the HMBC spectrum, the ¹H–¹³C long-range correlations from 2-H to an olefinic carbon C-3 (δ_C 161.6) and two quaternary carbons

¹Biomedical Information Research Center (BIRC), Japan Biological Informatics Consortium (JBIC), Koto-ku, Tokyo, Japan; ²NITE Biotechnology Development Center (NBDC), Department of Biotechnology, National Institute of Technology and Evaluation (NITE), Kisarazu, Chiba, Japan and ³Biomedical Information Research Center (BIRC), National Institute of Advanced Industrial Science and Technology (AIST), Koto-ku, Tokyo, Japan
Correspondence: Dr K Shin-ya, Biomedical Information Research Centre (BIRC), National Institute of Advanced Industrial Science and Technology (AIST), 2-4-7 Aomi, Koto-ku, Tokyo 135-0064, Japan.

E-mail: k-shinya@aist.go.jp or Dr M Takagi, Biomedical Information Research Centre (BIRC), Japan Biological Informatics Consortium (JBIC), 2-4-7 Aomi, Koto-ku, Tokyo 135-0064, Japan.

E-mail: motoki-takagi@aist.go.jp

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Figure 1 (a) Structure of **1**. (b) Key correlations of ^1H - ^1H double-quantum filtered COSY (bold line) and HMBC (arrow, proton to carbon) of **1**. (c) NOE observation for **1** (dashed arrow).

C-4 (δ_{C} 76.9) and C-9b (δ_{C} 59.6); from 10- H_3 to C-2 (δ_{C} 125.6), C-3 and C-4; from the methyl protons 11- H_3 (δ_{H} 1.36) to C-3, C-4 and an acetal carbon C-4a (δ_{C} 106.7); and from the methyl protons 20- H_3 (δ_{H} 1.28) to a carbonyl carbons C-1 (δ_{C} 194.9), C-4a and C-9b revealed a trimethylcyclohexenone ring (Figure 1b).

The long-range couplings from the methyl protons 12- H_3 (δ_{H} 1.45) to an oxygenated quaternary carbon C-5a (δ_{C} 80.3), an oxygenated olefinic carbon C-6 (δ_{C} 162.5) and a methine carbon C-9a (δ_{C} 54.1);

Table 1 ^{13}C - and ^1H -NMR data for **1**

Position	δ_{C}	δ_{H} (multiplicity, J in Hz)
1	194.9	
2	125.6	5.64 (br s)
3	161.6	
4	76.9	
4a	106.7	
5a	80.3	
6	162.5	
7	111.9	
8	190.9	
9	100.4	
9a	54.1	3.65 (s)
9b	59.6	
10	18.2	2.01 (d, 1.0)
11	24.4	1.36 (s)
12	25.8	1.45 (s)
13	6.8	1.63 (s)
14	170.8	
15	120.2	6.38 (d, 15.0)
16	139.6	7.30 (dd, 15.0, 11.0)
17	131.1	6.28 (ddd, 15.0, 11.0, 1.0)
18	137.4	6.11 (dq, 15.0, 7.0)
19	18.78	1.87 (dd, 7.0, 1.0)
20	18.80	1.28 (s)
14-OH		16.43 (s)

NMR spectra were taken on a Varian NMR System 500 NB CL (Varian, Palo Alto, CA, USA) in chloroform- d with the residual solvent peak as an internal standard (δ_{C} 77.0, δ_{H} 7.26 ppm).

from the methyl protons 13- H_3 (δ_{H} 1.63) to C-6, an olefinic carbon C-7 (δ_{C} 111.9) and a carbonyl carbon C-8 (δ_{C} 190.9); and from 9a- H (δ_{H} 3.65) to C-5a, C-6, C-8 and an olefinic carbon C-9 (δ_{C} 100.4) established dimethylcyclohexenone ring. In addition, a hydrogen-bonded hydroxyl proton 14-OH (δ_{H} 16.43) long-range coupled with three olefinic carbons C-9, C-14 (δ_{C} 170.8) and C-15 (δ_{C} 120.2) and a sequence from an olefinic proton 15- H (δ_{H} 6.38) to the methyl protons 19- H_3 (δ_{H} 1.87) through three olefinic protons 16- H (δ_{H} 7.30), 17- H (δ_{H} 6.28) and 18- H (δ_{H} 6.11) determined the hydroxyhexatriene side chain to connect at C-9 of the dimethylcyclohexenone ring. The stereochemistry of the olefins at C-15 and C-17 was determined as *trans* by their coupling constants ($J=15.0$ Hz). Finally, long-range couplings from the methine protons 9a- H to C-1, C-9b and C-20 (δ_{C} 18.80) and from 20- H_3 to C-9a elucidated the connectivity between the two cyclohexenone rings. Thus, the planar structure of **1** was determined as shown in Figure 1a.

The relative configuration was assigned on the basis of the analysis of NOE spectra. The NOE (Figure 1c) between 2- H and 13- H_3 , between 9a- H and 12- H_3 , between 9a- H and 20- H_3 and between 12- H_3 and 20- H_3 indicated that the hydroxyl groups at C-4a, the hydrogen atom 9a- H and the two methyl groups C-12 and C-20 showed the same direction on the furan ring as shown in Figure 1c.

We herein isolated a new sorbicillin derivative together with three known dimeric sorbicillin derivatives. Some homodimeric analogs of sorbicillin, such as bisorbibutenolide,¹⁰ bisvertinolone¹¹ and trichodimerol,¹² which were isolated from fungi *Penicillium* spp., *Trichoderma* spp. or *Verticillium* spp., have been reported. On the other hand, only two sorbicillin derivatives, that is, sorbicillactones A and B, have been reported as heterodimeric compounds that consist of sorbicillin and

alanine fumaramide units as secondary metabolites of marine sponge-derived fungus *P. chrysogenum*.¹⁵ The new sorbicillinoid, **1**, may be produced by cyclization between sorbicillin and the trihydroxyquinol antibiotic, KS 506p,¹⁶ as precursors.

It has been reported that sorbicillin derivatives are radical scavengers.¹⁶ Therefore, to evaluate the activity of **1** as a radical scavenger, we examined its inhibitory effect on L-glutamate toxicity in neuronal hybridoma N18-RE-105 cells, which can assess the radical scavenging activity.^{17–20} The protective activity of **1**, bisorbibutenolide, bisvertinolone and trichodimerol against L-glutamate toxicity in N18-RE-105 cells was tested. Compound **1**, bisorbibutenolide and bisvertinolone reduced L-glutamate toxicity in N18-RE-105 cells with EC₅₀ values of 71, 61 and 49 μ M, respectively; however, trichodimerol showed weak activity (EC₅₀ > 100 μ M).

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- 1 Blunt, J. W. *et al.* Marine natural products. *Nat. Prod. Rep.* **26**, 170–244 (2009).
- 2 Molinski, T. F., Dalisay, D. S., Lievens, S. L. & Saludes, J. P. Drug development from marine natural products. *Nat. Rev. Drug Discov.* **8**, 69–85 (2009).
- 3 Wagner-Döbler, I., Beil, W., Lang, S., Meiners, M. & Laatsch, H. Integrated approach to explore the potential of marine microorganisms for the production of bioactive metabolites. *Adv. Biochem. Eng. Biotechnol.* **74**, 208–238 (2002).
- 4 Blunt, J. W. *et al.* Marine natural products. *Nat. Prod. Rep.* **26**, 170–244 (2009).
- 5 Saleem, M. *et al.* Marine natural products of fungal origin. *Nat. Prod. Rep.* **24**, 1142–1152 (2007).
- 6 Motohashi, K. *et al.* New sesquiterpenes, JBIR-27 and -28, isolated from a tunicate-derived fungus, *Penicillium* sp. SS080624Scf1. *J. Antibiot.* **62**, 247–250 (2009).
- 7 Motohashi, K., Inaba, S., Takagi, M. & Shin-ya, K. JBIR-15, a new aspochracin derivative, isolated from a sponge-derived fungus, *Aspergillus sclerotiorum* Huber Sp080903f04. *Biosci. Biotechnol. Biochem.* **73**, 1898–1900 (2009).
- 8 Izumikawa, M. *et al.* JBIR-37 and -38, novel glycosyl benzenediols, isolated from the sponge-derived fungus, *Acremonium* sp. SpF080624G1f01. *Biosci. Biotechnol. Biochem.* **73**, 2138–2140 (2009).
- 9 Zhang, D. H. *et al.* Redoxcitrinin, a biogenetic precursor of citrinin from marine isolate of fungus *Penicillium* sp. *J. Microbiol. Biotechnol.* **17**, 865–867 (2007).
- 10 Abe, N., Murata, T. & Hirota, A. Novel oxidized sorbicillin dimers with 1,1-diphenyl-2-picrylhydrazyl-radical scavenging activity from a fungus. *Biosci. Biotechnol. Biochem.* **62**, 2120–2126 (1998).
- 11 Trifonov, L. S. *et al.* Bisvertinols—a new group of dimeric vertinoids from *Verticillium intertextum*. *Tetrahedron* **42**, 3157–3179 (1986).
- 12 Andrade, R., Ayer, W. A. & Mebe, P. P. The metabolites of *Trichoderma longibrachiatum*. 1. Isolation of the metabolites and the structure of trichodimerol. *Can. J. Chem.* **70**, 2526–2535 (1992).
- 13 Sassa, T., Aoki, H., Namiki, M. & Munakata, K. Plant growth promoting metabolites of *Sclerotinia sclerotiorum* part I. Isolation and structures of sclerotinin A and B. *Agric. Biol. Chem.* **32**, 1432–1439 (1968).
- 14 Furihata, K. & Seto, H. Constant time HMBC (CT-HMBC), a new HMBC technique useful for improving separation of cross peaks. *Tetrahedron Lett.* **39**, 7337–7340 (1998).
- 15 Bringmann, G. *et al.* The first sorbicillinoid alkaloids, the antileukemic sorbicillactones A and B, from a sponge-derived *Penicillium chrysogenum* strain. *Tetrahedron* **61**, 7252–7265 (2005).
- 16 Hirota, A., Morimitsu, Y. & Hojo, H. New antioxidative indophenol-reducing phenol compounds isolated from the *Mortierella* sp. fungus. *Biosci. Biotechnol. Biochem.* **61**, 647–650 (1997).
- 17 Kim, W. G. *et al.* New diphenazines with neuronal cell protecting activity, phenazostatins A and B, produced by *Streptomyces* sp. *J. Antibiot.* **50**, 715–721 (1997).
- 18 Murakami, Y. *et al.* Formobactin, a novel free radical scavenging and neuronal cell protecting substance from *Nocardia* sp. *J. Antibiot.* **49**, 839–845 (1996).
- 19 Shin-ya, K. *et al.* Carquinostatin B, a new neuronal cell-protecting substance produced by *Streptomyces exfoliatus*. *Biosci. Biotechnol. Biochem.* **61**, 1768–1769 (1997).
- 20 Shin-ya, K., Shimizu, S., Kunigami, T., Furihata, K. & Seto, H. A new neuronal cell protecting substance, lavanduquinocin, produced by *Streptomyces viridochromogenes*. *J. Antibiot.* **48**, 574–578 (1995).