The Journal of Antibiotics (2010) 63, 203–205

www.nature.com/ja

NOTE

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

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The Journal of Antibiotics (2010) **63,** 203–205; doi:10.1038/ja.2010.19; published online 5 March 2010

Keywords: *Penicillium citrinum*; radical scavenger; sorbicillin; sorbicillinol

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-15[7] 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] trichodimerol[12] 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. Me_2CO. After concentration *in vacuo*, the aqueous concentrate was extracted with EtOAc. The obtained organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The dried residue was washed with *n*-hexane–$CHCl_3$ (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 $CHCl_3$–MeOH (100:0, 99:1, 98:2, 95:5, 90:10 and 50:50, successively). The $CHCl_3$-eluate afforded redoxcitrinin, whereas the $CHCl_3$–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 $CHCl_3$–MeOH (98:2)-eluate (134 mg) was purified by preparative reversed-phase HPLC using an XBridge Prep C_{18} column (5.0 μm OBD, 19 i.d. ×150 mm; Waters, Milford, MA, USA) developed with 55% MeOH–H_2O (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 ($[\alpha]_D^{27}$ –350°, *c* 0.1, in MeOH; UV λ_{max} 221, 263 and 387 nm, in MeOH), and its molecular formula was determined to be $C_{23}H_{28}O_7$ by HR-ESI-MS (*m/z* 417.1910, $(M+H)^+$; calcd for $C_{23}H_{29}O_7$, 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 ^{13}C and ^{1}H 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 HMBC[14] spectrum as follows.

An allylic coupling between the methyl protons 10-H_3 (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 ^{1}H–^{13}C long-range correlations from 2-H to an olefinic carbon C-3 (δ_C 161.6) and two quaternary carbons

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Received 7 December 2009; revised 16 February 2010; accepted 17 February 2010; published online 5 March 2010

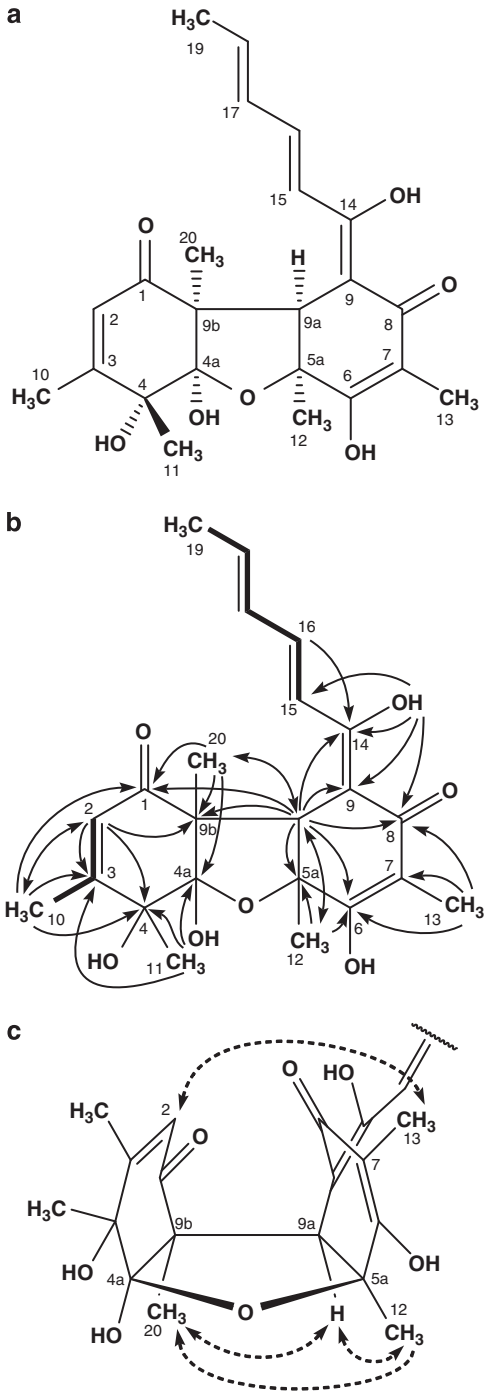

Figure 1 (**a**) Structure of **1**. (**b**) Key correlations of ^{1}H-^{1}H double-quantum filtered COSY (bold line) and HMBC (arrow, proton to carbon) of **1**. (**c**) NOE observation for **1** (dashed arrow).

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 ^{1}H-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] bisvertinolone[11] 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 EC_{50} values of 71, 61 and 49 μM, respectively; however, trichodimerol showed weak activity ($EC_{50} > 100$ μM).

ACKNOWLEDGEMENTS

This work was supported by a grant from the New Energy and Industrial Technology Department Organization (NEDO) of Japan and a Grant-in-Aid for Scientific Research (20380070 to KS) from The Japan Society for the Promotion of Science (JSPS), The Ministry of Education, Culture, Sports, Science and Technology (MEXT).

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