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NOTE

JBIR-124: a novel antioxidative agent from a marine sponge-derived fungus *Penicillium citrinum* SpI080624G1f01

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Active oxygen species cause many diseases such as atherosclerosis, inflammation, ischemia-reperfusion injury, rheumatoid arthritis and central nervous diseases.[1] Furthermore, senility and cancer initiation and progression are also believed to involve active oxygen species.[2] Thus, it is expected that effective antioxidative agents may prevent the onset and development of these diseases. In the course of our screening program for novel antioxidants, we have already reported the isolation of a new sorbicillinoid derivative, designated JBIR-59,[3] from cultures of *Penicillium citrinum* SpI080624G1f01 isolated from marine sponges collected offshore of Ishigaki Island, Okinawa Prefecture, Japan. Further screening of the cultures resulted in the isolation of a new JBIR-59 derivative designated as JBIR-124 (**1**, Figure 1). This paper describes the isolation, structure elucidation and brief biological activity of **1**.

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 (320 r.p.m.) at 27 °C for 2 days. Aliquots (5 ml) of the culture were transferred to 100-ml Erlenmeyer flasks containing 3 g of brown rice (Hitomebore, Miyagi, Japan), 6 mg of Bacto-yeast extract (BD Biosciences), 3 mg of sodium tartarate, 3 mg of potassium hydrogen phosphate and 9 ml of water and were incubated in static culture at 27 °C for 14 days.

The mycelia (gathered from 50 flasks) was extracted with 80% aqueous Me_2CO (1400 ml) and filtered. After removal of Me_2CO from the extract, the aqueous concentrate was extracted with EtOAc (1 liter×3). The obtained organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The dried residue (4.9 g) was subjected to normal-phase medium-pressure liquid chromatography (Purif-Pack SI-30, Shoko Scientific Co., Yokohama, Japan) and eluted with a gradient system of *n*-hexane–EtOAc (0–25% EtOAc) followed by the stepwise solvent system of $CHCl_3$–MeOH (0, 2, 5, 10, 20, 30 and 100% MeOH). The 5% MeOH eluate (534 mg) was applied on a Sephadex LH-20 column (GE Healthcare BioSciences AB, Uppsala, Sweden) and eluted with $CHCl_3$–MeOH (1:1) to obtain crude **1** (143 mg). The active eluate was purified by preparative reversed-phase HPLC using a CAPCELL PAK C18 MGII column (5.0 μm, 20 i.d.×150 mm; Shiseido, Tokyo, Japan) with 60% aqueous MeOH containing 0.1% formic acid (flow rate, 10 ml min^{-1}) to yield **1** (2.9 mg, retention time (Rt), 27.2 min), along with JBIR-59 (14.6 mg, Rt, 19.3 min) and bisorbibutenolide[4] (2.6 mg, Rt, 32.8 min).

Compound **1** was a yellow amorphous solid: $[\alpha]_D^{22}$ −282° (MeOH; *c* 0.15); UV λ_{max} nm (log ε) in MeOH: 232 (4.11), 267 (4.09), 307 (3.95) and 385 (4.05); HR-ESI-MS: *m/z*=431.2086 $[M+H]^+$, calculated for $C_{24}H_{31}O_7$ 431.2070. The IR absorption (ν_{max} 1656 cm^{-1}) of **1** indicated the presence of an unsaturated ketone function. The direct connectivity between each proton and carbon was established by the heteronuclear single-quantum coherence (HSQC) spectrum. The ^{13}C and ^{1}H NMR spectral data for **1** are listed in Table 1. A planar structure was established by analyses of the double quantum-filtered (DQF)-COSY spectrum together with the constant time HMBC[5] spectrum as follows.

The DQF-COSY spectrum appeared as consecutive correlations from the terminal methyl protons H_3-20 (δ_H 1.88) to an olefinic methine proton H-16 (δ_H 6.43) through three olefinic protons. In the HMBC spectrum, ^{1}H–^{13}C long-range correlations from singlet methyl protons H_3-14 (δ_H 1.59) to an oxygenated olefinic carbon C-6 (δ_C 167.6), a quaternary olefinic carbon C-7 (δ_C 110.7) and an α,β-unsaturated ketone C-8 (δ_C 192.2); from methyl protons H_3-13 (δ_H 1.42) to an oxygenated quaternary carbon C-5a (δ_C 80.0), a methine carbon C-9a (δ_C 55.1) and C-6; from a singlet methine proton H-9a (δ_H 3.63) to C-5a, C-6, C-8 and an olefinic carbon C-9 (δ_C 101.6), established a 1,3,4-trioxygenated dimethylcyclohexenone ring. In addition, the correlations from the olefinic proton H-16 to C-9 and an oxygenated olefinic

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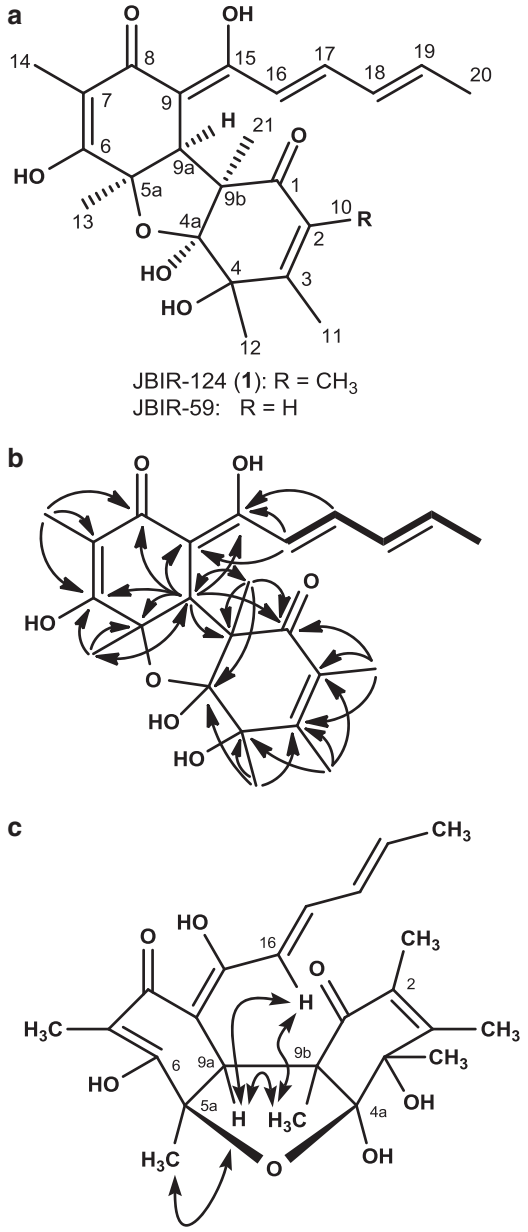

Figure 1 (**a**) Structure of JBIR-59 and **1**. (**b**) Key correlations of double quantum-filtered-COSY (bold line) and HMBC (arrow, proton to carbon) of **1**. (**c**) NOESY correlations of **1** (arrow).

carbon C-15 (δ_C 169.5), which long-range coupled in turn to the methine proton H-9a, indicated that an olefinic chain motif (from C-16 to C-20) was connected to C-15 as shown in Figure 1b.

Furthermore, correlations from singlet methyl protons H_3-12 (δ_H 1.28) to an olefinic quaternary carbon C-3 (δ_C 156.6), an oxygenated carbon C-4 (δ_C 76.6) and a hemiketal carbon C-4a (δ_C 106.1); from an allylic methyl signal H_3-11 (δ_H 1.97) to 2 olefinic carbons C-2 (δ_C 131.0) and C-3, and C-4; from another singlet methyl proton H_3-10 (δ_H 1.55) to an α,β-unsaturated ketone carbon C-1 (δ_C 196.4), C-2 and C-3; from a remaining singlet methyl signal H_3-21 (δ_H 1.26) to C-1, C-4a and a quaternary carbon C-9b (δ_C 59.8) revealed a tetramethylhexenone ring. The connection between C-9a and C-9b was revealed by the correlations from H_3-21 to C-9a. According to the 10 index of hydrogen deficiency deduced from the molecular formula of

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

Position	δ_C	δ_H(*multiplicity, J in Hz*)
1	196.4	
2	131.0	
3	156.6	
4	76.6	
4a	106.1	
5a	80.0	
6	167.6	
7	110.7	
8	192.2	
9	101.6	
9a	55.1	3.63 (s)
9b	59.8	
10	12.3	1.55 (s)
11	14.6	1.97 (s)
12	24.6	1.28 (s)
13	26.3	1.42 (s)
14	7.9	1.59 (s)
15	169.5	
16	120.9	6.43 (d, 15.0)
17	139.3	7.23 (dd, 11.4, 15.0)
18	131.6	6.29 (dd, 11.4, 15.0)
19	137.3	6.10 (dq, 6.6, 15.0)
20	18.8	1.88 (d, 6.6)
21	19.5	1.26 (s)

NMR spectra were taken on a Varian NMR System 600 NB CL in $CHCl_3$-*d*/MeOH-d_4 (1:1) with the residual solvent peak as an internal standard (δ_C 49.0, δ_H 3.35 p.p.m.).

1, the hemiketal carbon C-4a should connect to the other oxygenated carbon to construct a heterocyclic structure through an ether bond. The ^{1}H and ^{13}C NMR chemical shifts of **1** were well matched with those of JBIR-59, except for the C-2 moiety, and revealed the structure of **1** as shown in Figure 1a.

The configuration of the conjugated moiety was deduced as 16*E*, 18*E* by the characteristic proton–proton coupling constants ($^{3}J_{16H-17H}$ =15.0 Hz and $^{3}J_{18H-19H}$=15.0 Hz), respectively. A *Z* geometry for C-9 was elucidated by NOESY correlations between H-16/H-9a and H-16/H_3-21. The NOESY correlations between H-9a/H_3-13 and H-9a/H_3-21 indicated that the hydroxy group at C-4a, the hydrogen atom H-9a, and the two methyl groups CH_3-13 and CH_3-21 were oriented in the same direction on the furan ring, as shown in Figure 1c.

We evaluated the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of **1**. A 384-well plate was used for the DPPH radical scavenging assay.[6] Compound **1** and α-tocopherol as a positive control were dissolved in dimethyl sulfoxide to form the stock solution (1 mM). We mixed 10 µl of 400 µM DPPH dissolved in EtOH, 2 µl of sample solution and 8 µl of phosphate-buffered saline buffer in a 384-well microplate. After 30-min incubation at room temperature, the absorbance was measured at 540 nm. Compound **1** demonstrated DPPH radical scavenging activity with an IC_{50} value of 30 µM, which was almost the same as that of α-tocopherol (IC_{50}=9.0 µM) and JBIR-59 (IC_{50}=25 µM).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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