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NOTE

JBIR-76 and JBIR-77, modified naphthoquinones from *Streptomyces* sp. RI-77

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Members of the class Actinobacteria have been extensively studied because of their ability to produce pharmaceutically useful compounds. In order to construct a natural product library for bioactivity screening, we isolated a diverse variety of actinomycetes from marine resources, including marine sponges and tunicates, and mangrove soils, as well as the conventionally utilized terrestrial soils. As a result, we discovered novel compounds such as the anti-influenza compound JBIR-68,[1] promothiocin derivatives JBIR-83 and -84,[2] naphthablin analogs JBIR-79 and -80,[3] and new angucyclines JBIR-90, -91, -92, -93 and -116.[4] Further screening resulted in the isolation of two new cytotoxic compounds, JBIR-76 (**1**) and JBIR-77 (**2**), in addition to the antioxidative naphthoquinone-like polyketide, designated JBIR-85,[5] from the culture of *Streptomyces* sp. RI-77 obtained from a soil sample collected in Okinawa Prefecture, Japan. This paper describes the fermentation, isolation, structural elucidation and brief biological activities of **1** and **2**.

Streptomyces sp. RI-77 was isolated from a soil sample collected in Shuri, Okinawa Island, Japan, and cultivated in 50-ml test tubes containing 15 ml of seed medium composed of 1.0% starch (Kosokagaku, Tokyo, Japan), 1.0% Polypepton (Nihon Pharmaceutical, Tokyo, Japan), 1.0% molasses (Dai-Nippon Meiji Sugar, Tokyo, Japan) and 1.0% meat extract (Extract Ehlrich, Wako Pure Chemical Industry, Osaka, Japan pH 7.2, adjusted before sterilization). The test tubes were shaken on a reciprocal shaker at 27 °C for 2 days (320 r.p.m.). Aliquots (2.5 ml) of the culture were transferred into 500-ml baffled Erlenmeyer flasks filled with 100 ml of a production medium consisting of 0.9% oatmeal (Quaker, Chicago, IL, USA), 0.15% malt extract (Becton, Dickinson and Company; Franklin Lakes, NJ, USA), 0.09% yeast extract (Becton, Dickinson and Company), 0.9% soluble starch (Kosokagaku), 1.1% 3-(*N*-morpholino)propanesulfonic acid (Nacalai Tesque, Kyoto, Japan), 1.0% D-glucose (Kanto Chemical, Tokyo, Japan), 12 mg $MgSO_4$, 30 mg NaCl and 150 mg $CaCO_3$ at pH 7.0 (adjusted before sterilization), and cultured on a rotary shaker (180 r.p.m.) at 27 °C for 5 days.

After fermentation, the fermentation broth (2 l) was centrifuged, and the pelleted mycelial cake was extracted with acetone (800 ml) two times. The mycelium was removed by filtration, and the acetone extract was evaporated under reduced pressure. The residual aqueous concentrate was partitioned between EtOAc and water (200 ml × 3). The separated organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue (510 mg) was applied on a normal-phase medium-pressure liquid column (Purif-Pack SI-30; Shoko Scientific, Yokohama, Japan), and the column was successively eluted using a hexane-EtOAc solvent system (0, 5, 10, 20 and 25% EtOAc) followed by a $CHCl_3$–MeOH solvent system (0, 2, 5, 10 and 20% MeOH). The 10% MeOH-eluted fraction (38.0 mg) was further purified by preparative RP-HPLC using a CAPCELL PAK C_{18} MGII column (5.0 μm, 20 i.d. × 150 mm; Shiseido, Tokyo, Japan) with a 2996 photodiode array detector (Waters, Milford, MA, USA) and a 3100 mass detector (Waters) developed with 55% aqueous MeOH (flow rate, 10 ml min^{-1}) to yield JBIR-76 (**1**, 1.5 mg, retention time (Rt) = 13.2 min) together with known compound JBIR-85[5] (0.4 mg, Rt = 19.5 min). The 5% MeOH-eluted fraction (70.5 mg) was purified by preparative RP-HPLC using a CAPCELL PAK C_{18} MGII column with 68% aqueous MeOH (flow rate: 10 ml min^{-1}) to yield JBIR-77 (**2**, 1.4 mg, Rt = 16.3 min).

JBIR-76 (**1**) was isolated as a colorless oil: $[\alpha]^{25}_{D}$ +46.0, (*c* 0.1, MeOH); UV λ_{max} nm (log ε): 327 (3.98) in MeOH; IR (attenuated total reflection) ν_{max} cm^{-1}: 3735, 1770 and 1650. The molecular formula of **1** as $C_{15}H_{14}O_7$ was determined by positive high-resolution-electrospray ionization (HRESI)-MS (*m/z* 307.0833, calculated for $C_{15}H_{15}O_7$, 307.0818). The structure of **1** was established on the basis of its double-quantum-filtered-COSY and constant-time heteronuclear multiple-bond correlation (CT-HMBC)[6] spectra (Figure 1b). The ^1H and ^{13}C NMR spectroscopic data for **1** are shown in Table 1.

The 10 sp^2 ^{13}C signals among 15, including two methoxy carbons, indicated that **1** consisted of a highly conjugated skeleton. The strong *meta*-couplings from an aromatic proton H-5 (δ_H 6.62) to aromatic carbons C-7 (δ_C 135.0) and C-8a (δ_C 110.6), in addition to ^1H-^{13}C long-range couplings to C-4a (δ_C 138.7) and C-6 (δ_C 158.3) observed in the CT-HMBC spectrum established a benzene ring moiety. The ^1H-^{13}C long-range coupling observed between a methoxy proton 7-OMe (δ_H 3.71) and a quaternary carbon C-7 revealed that this methoxy group was substituted at the position of C-7. The ^1H-^{13}C long-range couplings from a phenolic hydroxy proton 8-OH (δ_H 13.20), which was considered to be hydrogen-bonded with a

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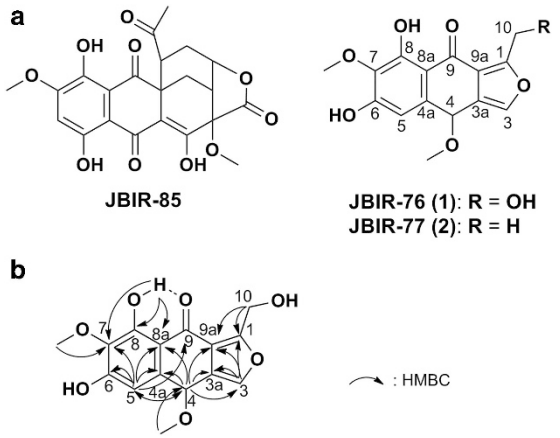

Figure 1 (**a**) Structures of JBIR-85, JBIR-76 (**1**) and −77 (**2**). (**b**) Correlations in CT-HMBC (arrows) spectra of **1**.

Table 1 ^{13}C (150 MHz) and ^{1}H (600 MHz) NMR spectroscopic data for JBIR-76 (1) in DMSO-d_6 and ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR spectroscopic data for JBIR-77 (2) in acetone-d_6

	1		*2*	
No.	δ_C	δ_H *(multiplicity, J, in Hz)*	δ_C	δ_H *(multiplicity, J, in Hz)*
1	160.0		159.0	
3	141.2	8.02, s	140.0	7.75, s
3a	122.1		123.0	
4	68.6	5.47, s	69.3	5.49, s
4a	138.7		139.3	
5	111.1	6.62, s	110.0	6.70, s
6	158.3		157.3	
7	135.0		135.3	
8	158.3		158.6	
8a	110.6		112.2	
9	186.0		187.3	
9a	116.5		116.7	
10	55.3	4.78, s	13.9	2.67, s
4-OMe	53.7	3.09, s	53.3	3.15, s
7-OMe	60.5	3.71, s	60.6	3.86, s
8-OH		13.20, s		13.46, s

NMR spectra were taken on Varian NMR System 600 NB CL and 500 NB LM (Agilent Technologies, Santa Clara, CA, USA). Chemical shifts were calibrated internally against the residual signal of the solvent in which the sample was dissolved (DMSO-d_6: δ_C 39.7, δ_H 2.49; acetone-d_6: δ_C 209.8, δ_H 2.04).

carbonyl carbon at the *peri* position, to aromatic carbons C-7, C-8 (δ_C 158.3) and C-8a established the penta-substituted benzene ring moiety. By taking into consideration its ^{13}C chemical shift at C-6 (δ_C 158.3), an oxygen atom was determined to be substituted at C-6. Further, the ^{1}H-^{13}C long-range couplings from a singlet hydroxymethyl proton H_2-10 (δ_H 4.78, δ_C 55.3) to aromatic carbons C-1 (δ_C 160.0) and C-9a (δ_C 116.5), and from an aromatic proton H-3 (δ_H 8.02) to C-1, C-3a (δ_C 122.1) and C-9a established a 2,3,4-trisubstituted furan moiety, and that a hydroxymethyl residue was substituted at the position of C-1. The ^{1}H-^{13}C long-range couplings from an oxymethine proton H-4 (δ_H 5.47) to C-3 (δ_C 141.2), C-3a, C-4a, C-5 (δ_C 111.1), C-8a and C-9a established a 6-6-5 ring system, as shown in Figure 1. The ^{1}H-^{13}C long-range coupling between a methoxy proton 4-OMe (δ_H 3.09) and C-4 (δ_C 68.6) revealed that the methoxy functional residue is substituted at the position of C-4. Finally, hydroxy groups at C-6 and C-10 were determined based on the molecular formula (Figure 1a).

JBIR-77 (**2**) was isolated as a colorless oil: $[\alpha]^{25}_D$ +27.8, *c* 0.1, MeOH; UV λ_{max} nm (log ε): 324 (3.98) in MeOH; IR (attenuated total reflection) ν_{max} cm^{-1} 3520, 1750 and 1650. The molecular formula of **2** as $C_{15}H_{14}O_6$ was determined by positive HRESI-MS (*m/z* 291.0863, calculated for $C_{15}H_{15}O_6$, 291.0869). The IR and UV spectra of **2** were similar to those of **1**. Most of the NMR spectroscopic data for **2** were also similar to those of **1** (Table 1). In the ^{1}H NMR spectrum of **2**, the singlet signal due to the oxymethylene group at H_2-10 in **1** was replaced with a singlet methyl proton (δ_H 2.67). In addition, the oxymethylene carbon in **1** was replaced with a methyl carbon (δ_C 13.9 for C-10) in **2**. These collective spectroscopic data proved **2** to be 10-dehydroxy **1** (Figure 1a).

The cytotoxic activities of **1** and **2** against human ovarian adenocarcinoma SKOV-3, malignant pleural mesothelioma Meso-1 and Jurkat cells were examined using a 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt (WST-8) colorimetric assay (Cell Counting Kit-8; Dojindo, Kumamoto, Japan). All cell lines were seeded in 384-well plates at a density of 1000 cells per 20 µl per well and incubated at 37 °C in a humidified incubator with 5% CO_2. After 72 h of treatment, **1** exhibited cytotoxic activities against SKOV-3, Meso-1 and Jurkat cells with IC_{50} values of 18.7, 11.3 and 7.6 µM, respectively. Compound **2** exhibited almost the same cytotoxic activities against SKOV-3, Meso-1 and Jurkat cells with IC_{50} values of 9.7, 33.8 and 3.6 µM, respectively.

In conclusion, we isolated two new modified naphthoquinones, JBIR-76 (**1**) and JBIR-77 (**2**), from the culture of *Streptomyces* sp. RI-77 obtained from a soil sample collected in Okinawa Prefecture, Japan. During the same period, the antioxidative naphthoquinone-like polyketide, designated JBIR-85,[5] was also isolated. The structures of **1** and **2** consisted of a new skeleton, which is structurally related to 5,8-dihydroxy-1-hydroxymethylnaphtho[2,3-c]furan-4.9-dione isolated from the roots of *Bulbine capitata*.[7] Biosynthetic studies of these compounds are now underway.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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