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NOTE

Isolation of a novel macrocyclic dilactone—JBIR-101—from *Promicromonospora* sp. RL26

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Actinomycetes have been the focus of extensive investigation, owing to their ability to produce pharmaceutically useful compounds. However, in recent years, the rate of discovery of novel compounds from these bacteria has decreased significantly.[1] As a consequence, it is now more important than ever to isolate actinomycetes from a wide variety of environmental sources, employing various isolation methods to obtain new bioactive compounds. Thus, we have focused on mangrove soil samples as a rich source of diverse actinomycetes in our efforts to isolate novel secondary metabolites. In the course of our screening program for cytotoxic compounds against malignant pleural mesothelioma, an aggressive neoplasm that develops from the pleura and is highly invasive to surrounding tissue,[2–4] we have already reported novel anti-malignant pleural mesothelioma compounds, namely, JBIR-23,[5,6] the teleocidin analogue JBIR-31,[7] the aminocaprophenone-alkaloid ficuseptamine B,[8] the 1,1-dichlorocyclopropane-skeleton-containing angucycline JBIR-88,[9] the angucycline analogues JBIR-90–93, -116[10] and the xanthoquinodin analogues JBIR-97-99.[11] As a result of further screening, we now disclose a new compound, termed JBIR-101 (**1**), from the culture of *Promicromonospora* sp. RL26, found in a mangrove soil sample (Figure 1a). This manuscript describes the fermentation, isolation, structural elucidation and a brief discussion of the biological activity of **1**.

The strain, *Promicromonospora* sp. RL26, was isolated from a mangrove soil sample collected in Nosoko, Ishigaki Island, Okinawa Prefecture, Japan. The strain was cultivated in 50 ml test tubes each containing 15 ml of a seed medium consisting of starch (Kosokagaku, Tokyo, Japan) 1.0%, polypepton (Nihon Pharmaceutical, Tokyo, Japan) 1.0%, molasses (Dai-Nippon Meiji Sugar, Tokyo, Japan) 1.0% and meat extract (Extract Ehlrich, Wako Pure Chemical Industry, Osaka, Japan) 1.0% (pH 7.2 before sterilization). The test tubes were shaken on a reciprocal shaker (355 r.p.m.) at 27 °C for 2 days. Aliquots (2.5 ml) of the broth were transferred to 500 ml baffled Erlenmeyer flasks containing 100 ml of a production medium containing starch (Kosokagaku) 1%, glucose 1%, glycerin (Nacalai Tesque, Kyoto, Japan) 1%, polypepton (Nihon Pharmaceutical) 0.5%, yeast extract (BD Biosciences, San Jose, CA, USA) 0.2%, corn steep liquor (Oriental Yeast, Tokyo, Japan) 1%, NaCl (Kanto Chemical, Tokyo, Japan) 0.1%, $CaCO_3$ (Kozaki Pharmaceutical, Tokyo, Japan) 0.32% and Sealife (Marinetech, Tokyo, Japan) 1.75% (pH 7.4 before sterilization), and cultured on a rotary shaker (180 r.p.m.) at 27 °C for 5 days.

The mycelia collected from the fermentation broth (2 l) by centrifugation were extracted with Me_2CO (400 ml). After removal of the acetone *in vacuo*, the remaining aqueous concentrate was partitioned with EtOAc (100 ml×3), and was further extracted with *n*-BuOH (100 ml×3). The *n*-BuOH layer was evaporated, and the dried residue (1230 mg) was subjected to reversed-phase medium-pressure liquid chromatography (Purif-Pack ODS-100, Shoko Scientific, Yokohama, Japan), using a H_2O–MeOH stepwise solvent system (0, 10 and 20% MeOH). The active eluate (10% MeOH, 36 mg) was further purified by preparative reversed-phase HPLC using a CAPCELL PAK C_{18} MGII (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 20% aqueous MeOH containing 0.1% formic acid (flow rate, 10 ml min^{-1}) to yield **1** (2.9 mg, retention time 32.5 min).

Compound **1** was obtained as a colorless amorphous powder. The molecular formula was determined by HR-ESI-MS (Waters LCT-Premier XE) to be $C_{20}H_{28}O_{16}$ (found: 523.1298 $[M–H]^-$, calcd: 523.1299) and the presence of hydroxy and ester groups deduced from the IR spectrum (ν_{max} (KBr) 3399, 1731 and 1286 cm^{-1}). The structure of **1** was determined by NMR spectral analyses. The direct connectivity of protons and carbons were established by heteronuclear single quantum coherence spectrum, and the

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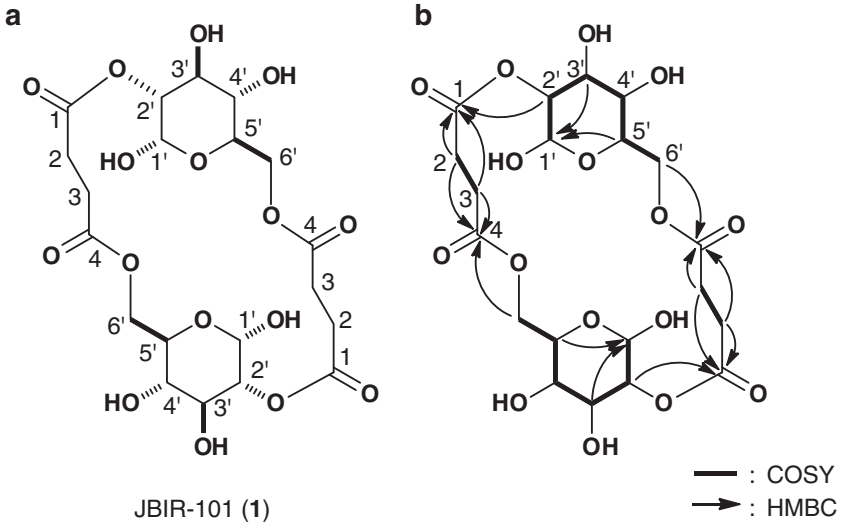

Figure 1 (**a**) Structure of JBIR-101 (**1**). (**b**) Key correlations in the DQF-COSY (bold lines) and CT-HMBC (arrows) spectra of **1**.

Table 1 ^{13}C **(150 MHz) and** ^{1}H **(600 MHz) NMR data for 1**

no.	δ_C	δ_H *(multiplicity, J in Hz)*
1	173.1	
2	30.5	2.59 (dt, 13.5, 8.8)
3	30.4	2.56 (dt, 15.2, 8.8)
4	170.0	
1′	96.9	5.08 (d, 4.2)
2′	75.0	4.31 (dd, 9.6, 4.2)
3′	70.7	3.55 (dd, 10.3, 9.6)
4′	70.4	3.11 (dd, 10.3, 9.7)
5′	72.0	3.83 (ddd, 9.7, 5.0, 2.2)
6′	63.7	4.41 (dd, 11.2, 5.0)
		4.00 (dd, 11.2, 2.2)

NMR spectra were obtained with the Varian NMR system 600 NB CL (Palo Alto, CA, USA) in DMSO-d_6, with the solvent peak used as an internal standard (δ_H 2.49 and δ_C 39.7 p.p.m.).

tabulated ^{13}C and ^{1}H NMR spectral data for **1** is shown in Table 1. The structure of **1** was elucidated in a series of DQF-COSY and constant time (CT)-HMBC[12] as follows.

By taking into consideration the number of carbon signals and molecular formula, **1** could be a symmetric structure. A ^{1}H–^{1}H correlation between two methylene protons 2-H (δ_H 2.59) and 3-H (δ_H 2.56) was observed in the DQF-COSY spectrum (Figure 1b), with the ^{1}H-^{13}C long-range couplings 2-H and 3-H to two carbonyl carbons C-1 (δ_C 173.1) and C-4 (δ_C 170.0), establishing a succinic acid moiety. The DQF-COSY and HMBC spectra also revealed the presence of a hexopyranose moiety (Figure 1b). The sugar unit was established by the sequence from oxymethine proton 1′-H (δ_H 5.08) to oxymethylene protons 6′-H (δ_H 4.41, 4.00) through four oxymethine protons 2′-H (δ_H 4.31), 3′-H (δ_H 3.55), 4′-H (δ_H 3.11) and 5′-H (δ_H 3.83), together with a long-range coupling between 5′-H and C-1′ (δ_C 96.9). Coupling constants among these protons revealed that the sugar moiety is an α-glucopyranoside. The direct ^{13}C-^{1}H coupling constant at C-1′ ($^{1}J_{C-H}$=170.2 Hz)[13] also supported the identification of an α-glucopyranoside moiety. The ^{1}H-^{13}C long-range couplings from oxymethine proton 2′-H to the carbonyl carbon C-1 and from the oxymethylene protons 6′-H to the carbonyl carbon C-4, in addition to the acylated shifts at 2′-H and 6′-H, proved that two succinic acid moieties and two glucopyranoside moieties are connected through ester bonds. By taking into consideration a symmetric structure, the macrocyclic diester structure of **1** was determined to be that depicted in Figure 1a.

We performed the alkaline hydrolysis of **1** to determine the absolute configuration of the sugar moiety. As a result, only one glucopyranose moiety was obtained as a sugar (1′-H (δ_C 93.4, δ_H 5.04, d, *J*=3.9 Hz), 2′-H (δ_C 71.1, δ_H 3.50, dd, *J*=10.0, 3.9 Hz), 3′-H (δ_C 72.7, δ_H 3.69, dd, *J*=10.0, 9.1 Hz), 4′-H (δ_C 69.8, δ_H 3.30, dd, *J*=9.7. 9.1 Hz), 5′-H (δ_C 72.4, δ_H 3.67, ddd, 9.7, 5.3, 2.7 Hz), 6′-H (δ_C 60.7, δ_H 3.71, dd, *J*=12.0, 2.7 Hz, δ_H 3.62, dd, *J*=12.0, 5.3 Hz) in D_2O). The optical rotation ($[\alpha]_D^{25}$+58.0° (*c* 0.02, H_2O) of this sugar residue was in accordance with that of D-glucopyranose (+52.7°).[14] Therefore, the structure of **1**, including absolute stereochemistry, was determined as shown in Figure 1a. Although macrocyclic dilactones composed of two acylsaccharides have been reported, namely cycloviracins B_1 and B_2,[15,16] and glucolipsins A and B,[17] this is a new type of symmetrical macrocyclic dilactones.

The cytotoxic activities of **1** against human malignant pleural mesothelioma ACC-MESO-1 cells[18] and human cervical carcinoma HeLa cells were estimated by 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium monosodium salt (WST-8) colorimetric assay (Cell Counting Kit, Dojiindo, Kumamoto, Japan) for 48 h. The novel isolated compound **1** exhibited cytotoxic activities against ACC-MESO-1 and HeLa cells with IC_{50} values of 48 μM and 37 μM, respectively.

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