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NOTE

A new cyclizidine analog—JBIR-102—from *Saccharopolyspora* sp. RL78 isolated from mangrove soil

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Actinomycetes have always attracted attention as potential candidates warranting extensive investigation, because of their ability to produce pharmaceutically useful compounds. However, in recent years, the rate of the discovery of novel compounds from these bacteria has decreased significantly.[1,2] As a consequence, it is now more important than ever to isolate actinomycetes from a wide variety of environmental sources and employ various isolation methods to obtain new bioactive compounds. In our efforts to isolate novel secondary metabolites, we have focused on mangrove soil as a rich source of diverse actinomycetes.

On the other hand, malignant pleural mesothelioma (MPM), which is associated with exposure to asbestos fibers, is an aggressive neoplasm developing from the pleura and is highly invasive to surrounding tissues.[3–5] MPM has been demonstrated to be resistant to all conventional therapies, including chemotherapy, radiotherapy and surgery, and the prognosis of patients remains very poor. Consequently, it is a tumor that continues to be a difficult clinical problem.[3–5] Therefore, development of novel therapeutic agents against MPM is strongly desired. In the course of our screening program for cytotoxic compounds against MPM cells, we have already discovered several novel anti-MPM compounds, namely, JBIR-23,[6,7] the teleocidin analog JBIR-31,[8] the aminocaprophenone-alkaloid ficuseptamine B,[9] the 1,1-dichlorocyclopropane-skeleton-containing angucycline JBIR-88,[10] the angucycline analogs JBIR-90-93 and -116,[11] the xanthoquinodin analogs JBIR-97-99,[12] and the macrocyclic dilactone JBIR-101.[13] As a result of further screening using MPM cells, we have discovered a new cyclizidine analog, termed JBIR-102 (**1**), from a culture of *Saccharopolyspora* sp. RL78,[14] found in a mangrove soil sample (Figure 1a). This manuscript describes the fermentation, isolation, structural elucidation and, briefly, the biological activity of **1**.

The strain *Saccharopolyspora* sp. RL78 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 1% starch (Kosoka-gaku, Tokyo, Japan), 1% Polypepton (Nihon Pharmaceutical, Tokyo, Japan), 1% molasses (Dai-Nippon Meiji Sugar, Tokyo, Japan) and 1% meat extract (Extract Ehlrich, Wako Pure Chemical Industry, Osaka, Japan) before sterilization, with the pH adjusted to 7.2. The test tubes were shaken on a reciprocal shaker (320 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 1% starch, 1% glucose, 1% glycerin (Nacalai Tesque, Kyoto, Japan), 0.5% Polypepton, 0.2% yeast extract (BD Biosciences, San Jose, CA, USA), 1% corn steep liquor (Oriental Yeast, Tokyo, Japan), 0.1% NaCl (Kanto Chemical, Tokyo, Japan), 0.32% $CaCO_3$ (Kozaki Pharmaceutical, Tokyo, Japan) and 1.9% marine art SF-1 (Tomita Pharmaceutical, Tokushima, Japan) before sterilization, with the pH adjusted to 7.4. The flasks were cultured on a rotary shaker (180 r.p.m.) at 27 °C for 5 days.

The mycelial cake collected from the fermentation broth (2 l) by centrifugation was extracted with Me_2CO (400 ml). After concentrating *in vacuo*, the residual aqueous concentrate was extracted with EtOAc (100 ml×3). The separated organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The dried residue (225 mg) was subjected to normal-phase medium-pressure liquid chromatography (Purif-Pack SI-30; Shoko Scientific, Yokohama, Japan), and was eluted stepwise by using a $CHCl_3$–MeOH solvent system (0, 2, 5 and 10% MeOH). The target eluate (5% MeOH, 29 mg) was further purified by preparative reversed-phase high-performance liquid chromatography, 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

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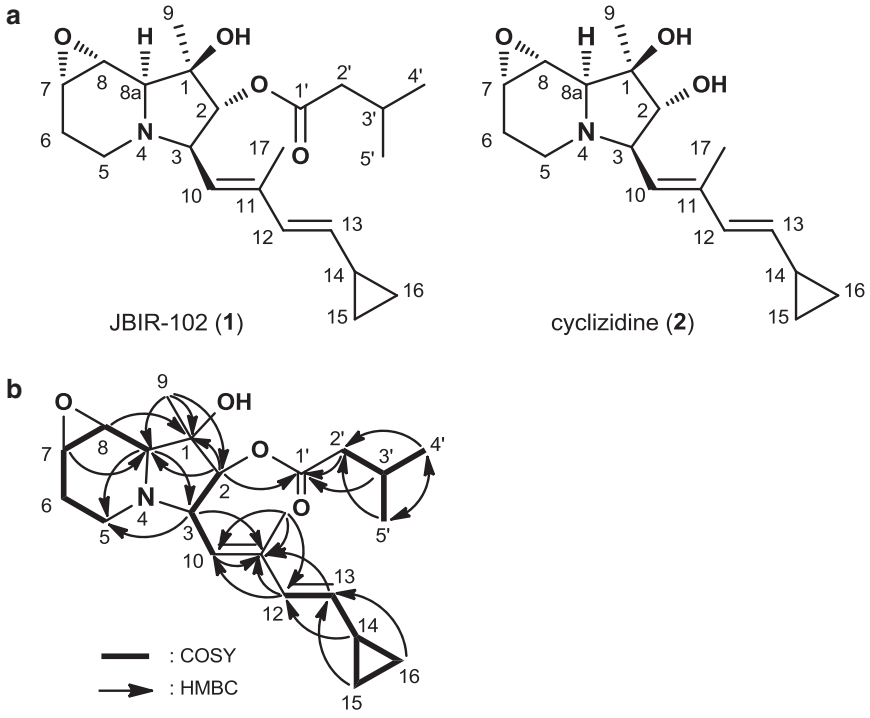

Figure 1 (**a**) Structures of JBIR-102 (**1**) and cyclizidine (**2**). (**b**) Correlations in DQF-COSY (bold lines) and CT-HMBC (arrows) spectra of **1**.

(Waters, Milford, MA, USA) and a 3100 mass detector (Waters) developed using 50% aqueous MeOH, containing 0.1% formic acid (flow rate, 10 ml min^{-1}) to yield **1** (3.4 mg, retention time 15.8 min).

Compound **1** was obtained as a colorless amorphous solid ($[\alpha]_D^{25}$ −52.7°, *c* 0.1, in MeOH; UV λ_{max} (ε) 243 (26 590) nm, in MeOH). The molecular formula was determined by high-resolution electrospray ionization–MS to be $C_{22}H_{33}NO_4$ (found: 376.2492 $[M+H]^+$, calcd: 376.2488). The presence of hydroxy and ester groups were deduced from its IR spectrum (ν_{max} (KBr) 3420, 1739, 1294 cm^{-1}). The direct connectivity between protons and carbons was established by a heteronuclear single quantum coherence spectrum, and the tabulated ^{13}C and ^{1}H NMR spectroscopic data for **1** is shown in Table 1. The structure of **1** was elucidated through the analysis of both DQF-COSY and constant time (CT)-HMBC[15] spectra as follows.

Four substructures were demonstrated through analyses of the DQF-COSY spectrum, and the connectivity of those 4 units was established by CT-HMBC. The sequence from methylene protons 5-H (δ_H 2.63, 1.87) to a methine proton 8a-H (δ_H 2.17) through methylene protons 6-H (δ_H 2.08) and oxymethine protons 7-H (δ_H 3.27) and 8-H (δ_H 3.41), which are considered to exist in an epoxide functional group from their ^{13}C chemical shifts (δ_C 52.3 and δ_C 52.4, respectively), was revealed by the DQF-COSY spectrum. A ^{1}H–^{13}C long-range coupling from the methine proton 8a-H to a methylene carbon C-5 (δ_C 44.0) helped to deduce that C-5 was bonded with C-8a (δ_C 72.2) through a heteroatom, which was determined to be a nitrogen atom from the ^{13}C chemical shift at C-5.

The proton spin systems of the oxymethine proton 2-H (δ_H 4.99, δ_C 79.9), an olefinic proton 10-H (δ_H 5.26, δ_C 125.2), and a heteroatom-bonded methine proton 3-H (δ_H 3.29, δ_C 63.9) were observed in the DQF-COSY spectrum. The ^{1}H–^{13}C long-range couplings from a singlet methyl proton 9-H (δ_H 1.47) to a quaternary oxygenated

Table 1 ^{13}C (150 MHz) and ^{1}H (600 MHz) NMR data for 1 and 2

	1		*2*	
No.	δ_C	δ_H *(multiplicity, J, in Hz)*	δ_C	δ_H *(multiplicity, J, in Hz)*
1	76.7		75.9	
2	79.9	4.99 (d, 7.6)	78.4	3.83 (d, 7.0)
3	63.9	3.29 (dd, 8.5, 7.6)	65.3	3.09 (dd, 9.2, 7.0)
5	44.0	2.63 (ddd, 10.6, 5.4, 2.2)	44.4	2.62 (ddd, 11.2, 5.3, 2.4)
		1.87 (dt, 10.6, 5.0)		1.86 (m)
6	26.4	2.08 (m)	26.1	2.07 (m)
7	52.3	3.27 (m)	52.2	3.28 (m)
8	52.4	3.41 (d, 4.1)	52.5	3.45 (d, 4.1)
8a	72.2	2.17 (br s)	72.7	2.09 (br s)
9	23.4	1.47 (s)	23.7	1.43 (s)
10	125.2	5.26 (d, 8.5)	125.1	5.36 (d, 9.2)
11	139.4		139.3	
12	132.9	6.18 (d, 15.6)	133.3	6.26 (d, 15.6)
13	135.0	5.24 (dd, 15.6, 9.7)	134.7	5.27 (dd, 15.6, 8.8)
14	15.0	1.48 (m)	15.0	1.48 (m)
15	7.6	0.78 (dd, 8.1, 1.7)	7.6	0.78 (dd, 7.8, 1.8)
16	7.5	0.42 (dd, 4.6, 1.7)	7.5	0.43 (dd, 4.4, 1.8)
17	13.1	1.73 (s)	13.3	1.78 (s)
1′	174.2			
2′	44.1	2.28 (dd, 14.8, 7.3)		
		2.26 (dd, 14.8, 7.0)		
3′	26.7	2.10 (m)		
4′	22.9	0.97 (d, 6.8)		
5′	22.8	0.96 (d, 6.8)		

NMR spectra were obtained using the Varian NMR system 600 NB CL (Palo Alto, CA, USA) in CD_3OD, and the solvent peak was used as an internal standard (δ_H 3.35 p.p.m. and δ_C 49.0 p.p.m.).

carbon C-1 (δ_C 76.7), the oxymethine carbon C-2 and the methine carbon C-8a, established the sequence from C-2 to C-8a through C-1. A long-range coupling from the methine proton 8a-H to a methine carbon C-3 (δ_C 63.9) determined a five-membered cyclic structure. Although the chemical shifts at C-3 and C-8a are greatly shifted downfield, these results indicate that this five-membered cyclic substructure is a pyrrolidine moiety. Taken together, **1** consists of a unique octahydroindolizine skeleton.

The sequence from an olefinic methine proton 12-H (δ_H 6.18) to a methylene proton 16-H (δ_H 0.42) through an olefinic proton 13-H (δ_H 5.24), a methine proton 14-H (δ_H 1.48) and a methylene proton 15-H (δ_H 0.78), was observed in the DQF-COSY spectrum. The ^{1}H–^{1}H spin coupling between 14-H and 16-H established a cyclopropane moiety. The ^{1}H–^{13}C long-range couplings from a methyl proton 17-H (δ_H 1.73) to the olefinic methine carbons C-10 (δ_C 125.2) and C-12 (δ_C 132.9), and an olefinic quaternary carbon C-11 (δ_C 139.4), revealed the connectivity between the vinylcyclopropane and the octahydroindolizine moieties through a vinyl methyl group. The geo-stereochemistry of this diene moiety was determined as 10*E* and 12*E*, respectively, according to the high-field-shifted chemical shift at a methyl carbon C-17 (δ_C 13.1) and a $J_{12H,13H}$=15.6 Hz.

A remaining substructure was elucidated as follows. The sequence from methylene protons 2′-H (δ_H 2.28, 2.26) to the methyl proton 4′-H (δ_H 0.97) through a methine proton 3′-H (δ_H 2.10), which was ^{1}H spin coupled to a methyl proton 5′-H (δ_H 0.96), was determined by analysis of the DQF-COSY spectrum. In the HMBC spectrum, the methylene protons 2′-H and the oxymethine proton 2-H were coupled to an ester carbonyl carbon C-1′ (δ_C 174.2). Thus, a 3-methylbutanoic acid moiety was shown to be substituted at the C-2 position in the octahydroindolizine skeleton through an ester bond (Figure 1b).

To determine the absolute configuration of the cyclizidine moiety, **1** was reacted with 0.2 N NaOH at 50 °C for 1 h to yield cyclizidine (**2**, Figure 1a). The structure of **2** was confirmed with NMR spectroscopic data (Table 1). The results imply that the NMR assignments of **2** by Freer *et al.*[16] are incorrect, and our data are identical to those reported by Leeper *et al.*[17] The obtained **2** showed an optical rotation value ($[\alpha]_D^{25}$ −48.3, *c* 0.1, MeOH) similar to the value ($[\alpha]_D^{25}$ −29.5, *c* 0.5, MeOH) of **2** reported by Hanessian *et al.*,[18] who proposed the absolute configuration of **2** by total synthesis of *ent*-cyclizidine. Thus, the absolute configuration of **1** was deduced to be the same as that of **2** (Figure 1a). Although an acetylcyclizidine has been reported,[16] this is the first report of an isobutyl ester derivative of cyclizidine.

Cytotoxic activities against human MPM ACC-MESO-1 cells[19] and human cervical carcinoma HeLa cells were determined by a colorimetric assay by using 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium monosodium salt (WST-8, Cell Counting Kit; Dojiindo, Kumamoto, Japan) for 48 h. Compounds **1** and **2** exhibited cytotoxic activities against ACC-MESO-1 with IC_{50} values of 39 μM and 32 μM and against HeLa cells with IC_{50} values of 29 μM and 16 μM, respectively.

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