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BRIEF COMMUNICATION

JBIR-150, a novel 20-membered polyene macrolactam from marine-derived *Streptomyces* sp. OPMA00071

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Abstract

During the course of constructing a natural product library for drug screening consisting of microbial culture extracts originated from marine samples, we evaluated natural product components profiles via UPLC TOF-MS and routine biological tests for cytotoxic and antibiotic activities for all of the culture extract samples. By combination of chemical screening and biological activities, we succeeded in discovering a 20-membered macrolactam antibiotic subsequently designated JBIR-150 (**1**) from a marine-derived actinomycete identified as *Streptomyces* sp. that was isolated from an Okinawan marine sediment. The chemical structure of **1** was determined by interpreting NMR spectroscopic and mass spectrometric data. Compound **1** exhibited moderate cytotoxicity against MESO-1 and Jurkat cells.

During the course of our screening program for new anticancer agents from Okinawan marine microorganisms, a new compound, designated JBIR-150 (**1**), was isolated from the culture broth of *Streptomyces* sp. OPMA00071. Chemical and spectroscopic analyses revealed that **1** belonged to the class of 20-membered polyene macrolactam antibiotics. Herein, we report the fermentation, isolation, structure elucidation, and preliminary biological activities of **1**.

Strain OPMA00071 was isolated from marine sediments collected in Okinawa prefecture, Japan. The isolated strain was identified as *Streptomyces* sp. on the basis on 99.9% similarity in the 16S rDNA gene sequence to *Streptomyces heliomycini*. The producing strain was cultivated in 50-ml test tubes, each containing 15 ml of a seed medium consisting of 1.0% starch, 1.0% Hipolypepton, 1.0% molasses, and 1.0% meat extract at pH 7.2 (adjusted before sterilization). 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 culture were transferred into 500-ml baffled Erlenmeyer flasks filled with 100 ml of a production medium consisting of 4 g β-cyclodextrin, 0.5 g glycerol, 2 g Pharmamedia, 0.5 mg $CuSO_4$•$5H_2O$, 0.5 mg $MnCl_2$•$4H_2O$, and 0.5 mg $ZnSO_4$•$7H_2O$, and cultured on a rotary shaker (180 r.p.m.) at 27 °C for 5 days.

The fermentation broth (2L) was centrifuged to obtain a mycelial cake, which was extracted with acetone (250 ml × 3). The extract was concentrated in vacuo, and the residual aqueous concentrate was successively washed with EtOAc followed by extraction with *n*-BuOH. The *n*-BuOH layer was then concentrated in vacuo. The dried residue (2.29 g) was triturated in MeOH (5 ml) and the residue was filtered using a Kiriyama-rohto device (Kiriyama, Tokyo, Japan) to afford JBIR-150 (**1**, 190.0 mg) as a colorless amorphous powder: $[\alpha]^{21}_D$ 163 (*c* 0.01, MeOH); UV λ_{max} nm (log ε): 227 (4.4), 260 (4.2), 310 (3.8), and 324 (3.7) in MeOH; IR (ATR) ν_{max} 3300 and 1630 cm^{-1} (hydroxy and amide carbonyl). Its molecular formula $C_{28}H_{39}NO_3$ was established by an $[M+H]^+$ ion at *m/z* 438.2977 (calcd for $C_{28}H_{40}NO_3$, 438.3003) in a high-resolution electrospray ionization mass spectrometry.

Because **1** was insoluble in CH_3OH, CH_3CN, *n*-hexane, EtOAc, $CHCl_3$, *n*-BuOH, and water, the NMR spectra were

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Table 1 ^{13}C and ^{1}H NMR spectroscopic data for JBIR-150 (**1**) in DMSO-d_6

Position	δ_C, Type	δ_H (Multiplicity, J in Hz)
1	165.9, C	0.88, d (7.2)
2	123.9, CH	5.79, d (15.0)
3	139.2, CH	6.65, dd (11.0, 15.0)
4	130.8, CH	6.19, dd (11.0, 15.0)
5	123.8, CH	6.14, ovl[a]
6	137.2, CH	5.91, ovl[a]
7	137.8, CH	5.44, dd (7.5, 15.0)
8	66.9, CH	4.53, dd (7.5, 7.5)
9	44.2, CH_2	1.44, ovl[a]; 1.35, ovl[a]
10	68.9, CH	3.83, m
11	38.3, CH_2	2.48, ovl[a]; 2.24, ovl[a]
12	126.2, CH	5.89, ovl[a]
13	136.7, CH	6.15, ovl[a]
14	133.6, C	
15	130.1, CH	5.87, ovl[a]
16	131.5, CH	6.27, dd (11.0, 15.0)
17	129.5, CH	5.39, ddd (5.5, 11.0, 15.0)
18	39.2, CH_2	2.32, m; 1.82ddd (11.0, 11.0, 11.0)
19	49.3, CH	3.74, m
20	38.7, CH_2	2.19, ovl[a]
21	129.1, CH	5.53, dd (7.0, 15.0)
22	132.4, CH	6.01, dd (11.0, 15.0)
23	130.9, CH	5.97, ovl[a]
24	132.8, CH	5.57, dd (7.0, 14.0)
25	34.5, CH_2	1.99, dd (7.0, 14.0)
26	22.4, CH_2	1.34, ovl[a]; 1.23, ovl[a]
27	14.0, CH_3	0.84, t (7.5)
28	13.4, CH_3	1.74, s

[a]Overlapped with other peaks. Measured on a 500 NB CL NMR spectrometer (Varian, Palo Alto, CA, USA) at 500 MHz for ^{1}H and 125 MHz for ^{13}C with the residual solvent peak as the internal standard (δ_H 2.49, δ_C 39.7 ppm)

measured in DMSO-d_6. The structure determination of **1** was executed based on a series of 2D NMR analyses such as double quantum filtered COSY (DQF-COSY), gradient-enhanced heteronuclear single quantum coherence with adiabatic pulses (HSQCAD), and gradient-selected heteronuclear multiple bond correlation using adiabatic pulses (HMBCAD). The ^{13}C and ^{1}H NMR data for **1** are listed in Table 1.

The well-resolved DQF-COSY spectrum of **1** indicated two partial structures. The first one is ranging from a doublet olefinic methine proton 2-H (δ_H 5.79) to an olefinic methine proton 13-H (δ_H 6.15) through olefinic methine protons 3-H (δ_H 6.65), 4-H (δ_H 6.19), 5-H (δ_H 6.14), 6-H (δ_H 5.91), and 7-H (δ_H 5.44), an oxymethine proton 8-H (δ_H 4.53), and aliphatic methylene protons 9-H_2 (δ_H 1.44, 1.35),

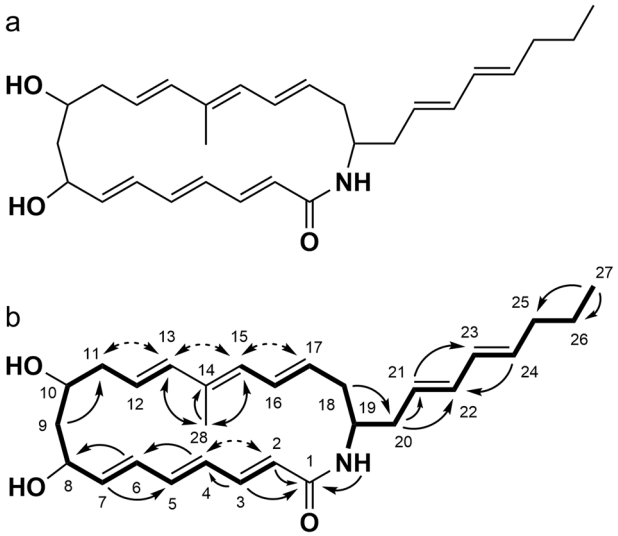

Fig. 1 **a** Structure of JBIR-150. **b** NMR analysis of JBIR-150. COSY: bold line; HMBC (^{1}H to ^{13}C): arrows, ROESY: dashed arrows

an oxymethine proton 10-H (δ_H 3.83), aliphatic methylene protons 11-H_2 (δ_H 2.48, 2.24) and an olefinic methine protons 12-H (δ_H 5.89). The second one from olefinic methine proton 15-H (δ_H 5.87) to terminal methyl protons 27-H_3 (δ_H 0.84) via olefinic methine protons 16-H (δ_H 6.27) and 17-H (δ_H 5.39), methylene protons 18-H_2 (δ_H 2.32, 1.82), a nitrogen-substituted methine proton 19-H (δ_H 3.74), methylene protons 20-H_2 (δ_H 2.19), olefinic methine protons 21-H (δ_H 5.53), 22-H (δ_H 6.01), 23-H (δ_H 5.97), and 24-H (δ_H 5.57), and aliphatic methylene protons 25-H_2 (δ_H 1.99) and 26-H_2 (δ_H 1.34, 1.23). In the HMBC spectrum, ^{1}H–^{13}C long-range couplings from singlet methyl protons 28-H_3 (δ_H 1.74) to olefinic quaternary carbon C-14 (δ_C 133.6) and olefinic methine carbons C-13 (δ_C 136.7) and C-15 (δ_C 130.1) connected C-13 and C-15 via C-14. ^{1}H–^{13}C Long-range couplings from olefinic protons H-13 and H-15 to C-28 also supported this connection. A macrolactam structure was established by the ^{1}H–^{13}C long-range couplings from the olefinic protons 2-H and 3-H and an amide proton (δ_H 7.39), which was spin coupled with 19-H, to an amide carbonyl carbon C-1 (δ_C 165.9). Based on the double bond equivalents of **1** and of the proton and carbon shifts, two hydroxy groups were substituted at the positions of C-8 and C-10. Thus, the gross structure of **1** was established as shown in Fig. 1.

The 2*E*, 4*E*, 6*E*, 12*E*, 14*E*, 16*E*, 21*E*, and 23*E* geometry was assigned based on the large $^{3}J_{H,H}$ values ($J_{2,3} = J_{4,5} = J_{6,7} = J_{16,17} = J_{21,22} = J_{23,24} = 14$–$15$ Hz) and ROESY correlations between 11-H_2 and 13-H, 13-H and 15-H, and 15-H and 17-H.

Structurally related compounds are the 20-membered polyene macrolactams heronamide C and F and 8-deoxyheronamide C[1–4] from a marine-derived

Streptomyces sp., which showed moderate cytotoxic activities. Compound **1** also exhibited cytotoxicity against human malignant mesothelioma MESO-1 and human T-lymphoma Jurkat cells [5], with IC_{50} values of 2.3 and 0.90 μM, respectively, and weak cytotoxic effects against human ovarian adenocarcinoma SKOV-3 at a concentration of 100 μM. Tests for further biological activities and biosynthetic studies of **1** are now underway.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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